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Honey bee nutritional health in agricultural landscapes: Relationships to pollen and habitat diversity

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Honey bee nutritional health in agricultural landscapes: Relationships to pollen and habitat diversity

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The student author and the program of study committee are solely responsible for the content of this dissertation. The Graduate College will ensure this dissertation is globally accessible and will not permit alterations after a degree is conferred.

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ABSTRACT

Managed honey bees are the most important pollinator worldwide, contributing to pollination of numerous crops, and they highly valued for their production of honey. The USA and Europe have experienced high colony losses in recent years, impeding the sustainable development of the beekeeping industry and endangering food safety due to its heavy reliance on insect pollination. The extensive production of crops across large areas of the U.S. and Europe have introduced multiple biotic and abiotic stressors for bees, including poor forage, pathogens and parasites, and pesticides, contributing to high colony losses. The Midwestern U.S., as a region with extensive monoculture-based agricultural production, has also been identified as a critical area for pollinator declines, making this area an important target area for honey bee health improvement.

For my doctoral research, I focused on honey bee health in the state of Iowa, at the epicenter of extensive agricultural production in the Midwestern U.S. I determined how the diversity and abundance of pollen, the main dietary source of proteins, lipids and micronutrients for honey bees, was affected by agricultural vs. natural foraging habitats, floral resources, and conservation practices (i.e. cropland integrated with strips of prairie vegetation). I also assessed whether the most common pollen types collected by honey bees in agricultural landscapes in central Iowa improved one aspect of honey bee health, resistance to virus infection. In addition, I used multi-year honey bee pollen collections to understand how annual weather fluctuations affect pollen collection. Finally, I determined if overall colony health would be improved with the integration of prairie strips into cropland as apiary sites.

My findings provided several novel insights into honey bee landscape nutrition in agroecosystems. First, I found that low cultivation landscapes (lower percentage of cropland)

did not improve pollen forage for honey bees compared to high cultivation landscapes (higher percentage of cropland). Legumes were the major source of pollen in agricultural landscapes, small increase in diversity of plants used as a source of pollen enhanced the survival of honey bees infected with viruses. Pollen collected by honey bees did not vary depending on land use types adjacent to honey bee colonies, with no marked differences between apiaries placed in soybean fields, diversified fruit and vegetable farms, and prairies. On a year-to-year basis, co-occurrence of drought and high temperature conditions had the potential to reduce pollen abundance available to honey bees. With respect to conservation habitat, restored prairies were important forage sources for honey bees, primarily in the late growing season period when crops and nonnative weedy plants ceased blooming. Integrating prairie plants into cropland (namely prairie strips, which are typically smaller than traditional prairie restorations or remnants) significantly improved pollen abundance and overall health of honey bee colonies across the growing season, compared to cropland without prairie strips. Overall, these results highlighted the potential for integrating beekeeping, crop production, and conservation practice, i.e. integrating native plants in to cropland, as a sustainable model for simultaneously enhancing honey bee health, agriculture, and biodiversity.

CHAPTER 1. GENERAL INTRODUCTION

Literature review

The Western honey bee, *Apis mellifera* L., is an eusocial colony-forming insect native to Europe, Africa, and the Middle East that is managed worldwide by beekeepers as a semi-domesticated species (Le Conte and Navajas 2008, Hung et al. 2018). Honey bees are the most important managed pollinator species, pollinating over 150 crops, and the annual value of the pollination attributed to honey bees is $ 15 billion in only USA (Thapa 2006, Calderone 2012). In addition to the pollination service provided by honey bees, honey is also an important food source with an estimated annual value as high as $ 1.25 billion across the world (vanEngelsdorp and Meixner 2010). As pollination occurs, honey bees collect nectar and pollen from flowers to satisfy their nutritional needs. Floral nectar is collected by nectar forager bees, processed by hive bees, and stored as honey, which is the main source of carbohydrate nutrition for honey bees and can be harvested by beekeepers for sale (Black 2006, Brodschneider and Crailsheim 2010, Wright et al. 2018). Nectar also contains trace amount of amino acids, fatty acids, minerals, vitamins and phytochemicals such as nicotine and alkaloids (Wright et al. 2018). The trace components in nectar may play a role in nectar attractiveness, but recent studies also suggest phyotchemicals in honey can stimulate honey bee detoxification responses and longevity (Liao et al. 2017). Pollen is collected by foragers bees, either immediately consumed or stored as semi-fermented “bee bread” in the hive and serves as the major source of dietary protein, lipid, and numerous micronutrients including minerals and vitamins (Black 2006, Wright et al. 2018). Pollen also contains a considerable amount of carbohydrate including starch, sugar and fiber, and in fact carbohydrates are usually the second most abundant macronutrient after protein (Kauffeld

1980, Black 2006), but pollen is still a minor provider of carbohydrate nutrition compared to honey stores.

Despite their important value as crop pollinators, in recent years managed honey bees in some agricultural regions (e.g. Europe and the USA) have experienced high colony losses that make sustainable beekeeping challenging (Leff et al. 2004, Foley et al. 2005, Potts et al. 2010, Fritz et al. 2015). A combination of biotic and abiotic stressors, including poor forage, pathogens and pests, and pesticides, contribute to high colony losses (Goulson et al. 2015). The modern expansion of agriculture and urban development reduces land cover in natural habitats with diverse floral resources, leading to poor forage (nectar and pollen) for honey bees including an overall forage shortage and/or declining forage diversity (Naug 2009, Goulson et al. 2015). Poor forage can reduce honey bees' resistance to pests, pathogens and insecticides (Schmehl et al. 2014, Dolezal and Toth 2018). Pests, pathogens and insecticides, in turn, can negatively reduce foraging abilities when honey bee health is compromised by those stressors (Schneider et al. 2012, Lach et al. 2015). Forage availability and use by honey bees are thus fundamental to honey bee health and represent the major focus of this dissertation. Therefore, I begin by summarizing studies documenting several key factors that are known to affect honey bee foraging behavior, foraging preference, and the collection of different forage components.

1. Colony nutritional status and population size drive foraging activity of honey bees.

Nutritional status and population size can affect foraging activity in honey bee colonies (Wolf and Schmid-Hempel 1990, Fewell and Winston 1992, Eckert et al. 1994). Typically, individual honey bee workers specialize on collecting pollen, nectar, water, or plant resins (propolis), with the majority of a colony's foragers usually concentrating on either nectar or pollen foraging (Wright et al. 2018). Low pollen stores and high brood amount are two factors that increase the number of pollen foragers assigned by colonies or increase pollen load size of

each pollen forager during pollen collection. Brood pheromone produced by brood stimulates pollen collecting activities (Pankiw et al. 1998, Pankiw 2004). Pollen foraging bees from colonies with larger brood and adult populations spend more time collecting pollen compared to colonies with smaller brood and adult populations. Low colony nectar stores stimulate more foragers to collect nectar (Schulz et al. 1998), but unlike pollen collection, nectar foragers from larger colonies carry lighter nectar loads during each foraging trip (Fewell et al. 1991). In addition, the age of forager bees can affect foraging activities, with unskilled new foraging bees collecting less forage than more experienced bees (Schippers et al. 2006).

2. Honey bee health affects foraging capability.

Parasites, pathogens and pesticides are important stressors contributing to declines in honey bee health, and each of these factors can negatively affect honey bees' foraging abilities. Under conditions of *Nosema* parasitization or virus infection, forager bees may carry less pollen during each foraging trip (Lach et al. 2015) or make fewer number of flights each day (Alaux et al. 2014), leading to less pollen collected (Anderson and Giacon 1992). Honey bees infected with *Nosema,* parasitized by *Varroa destructor* (Varroa mite), or infected with viruses are more likely to fail to return to their home colonies compared to healthy bees without parasites or viruses (Kralj and Fuchs 2010, Li et al. 2013). These pests can interact; for example, Varroa mites are a major vector of viruses (Ramsey et al. 2019), widely recognized as the most damaging pest for beekeeping in the USA, contributing in a major way to colony losses (Steinhauer et al. 2018, Haber et al. 2019). Varroa mites feed on the hemolymph and fat body of developing brood and adult bees, compromising their health, and making them more susceptible to pathogen infection (Ramsey et al. 2019).

Neonicotinoids are one of widely used classes of insecticides and have been commonly blamed for causing colony losses, but the frequency of neonicotinoid residues found in nectar

and pollen is generally low compared to many other insecticides (Mullin et al. 2010, Blacquière et al. 2012, Fairbrother et al. 2014, Godfray et al. 2014, Long and Krupke 2016). Neonicotinoids are known to reduce worker bee flight frequency and the number of foragers recruited for foraging (Bortolotti et al. 2003, Colin et al. 2004, Ramirez-Romero et al. 2005, Schneider et al. 2012). Neonicotinoids also increase time spent on each foraging trip, making foraging activity less efficient (Schneider et al. 2012). Neonicotinoids can also increase the chance of failure in homing behavior, possibly making returns back to the hive more difficult/unlikely for foragers (Bortolotti et al. 2003, Yang et al. 2008, Henry et al. 2012). Neonicotinoids also impair learning and memory abilities of honey bees (Decourtye et al. 2001, Decourtye et al. 2003, Decourtye et al. 2004, Zhang and Nieh 2015) which could reduce foragers' success in finding and collecting forage. Besides neonicotinoids, other insecticides are frequently found in honey bee forage (Long and Krupke 2016) and can also reduce honey bee foraging activities, for example deltamethrin (Vandame et al. 1995, Ramirez-Romero et al. 2005). Due to negative impacts on foraging activities, insecticides have the potential to reduce food collected by colonies and increase mortality of adult bees, possibly contributing to colony collapse.

3. Changing weather and climate affect foraging activity.

Daily changes in temperature affect honey bee foraging activity (Abou-Shaara 2014, Apiculture-Bulletins 2015). Bees are inactive when the ambient temperature is below minimum flight temperature (8 °C) or above optimum (30 °C) flight temperature, and colony foraging activity is usually low in the early morning, such as at 6 - 8 am (Apiculture-Bulletins 2015, Islam et al. 2015). The most active foraging time can switch from morning to afternoon, based on types of plants available as forage (Butler 1945, Holst and Nansen 2002, Evans and Spivak 2006, Baum et al. 2011, Silva et al. 2013, Shackleton et al. 2016), as well as the daily timing of when plants produce the most abundant pollen (Galán et al. 1991).

Annual weather fluctuations can affect the amount of pollen produced by plants (Bonny 1980, Nilsson and Persson 1981, O'Neal and Waller 1984, Galán et al. 1991, Emberlin et al. 1993), thus possibly affecting forage availability for honey bees. In addition to reducing the amount of nectar and pollen in flowers available to be collected, inclement weather events such as rainfall disturb foraging activities and are associated with food shortage (Bilisik et al. 2008, De Novais et al. 2009). Though we lack of scientific studies to determine the effect of drought on honey bees' foraging activities, evidence shows that drought can impede plant growth and production of nectar and pollen, contributing to decline in quantity and diversity of forage (Le Conte and Navajas 2008, Antúnez et al. 2015, Thomson and Irwin 2016, Garavito 2017). Under the worst scenario, prolonged unfavorable weather may cause colonies to starve if food stores run out. Starvation is more likely to happen at specific times, such as early spring when colony food stores are already low post-overwintering, while at the same time environmentally available floral resources are relatively low, and temperatures and weather conditions variable for foraging activity (Lecocq et al. 2015). In addition to direct negative effects of unfavorable weather on foraging activities, unfavorable weather may also increase the occurrence and contamination of pathogens and insecticide, thus indirectly affecting foraging activities. For example, cold temperatures in spring can also increase the occurrence of pathogens or parasites such as *Nosema apis* and *Ascosphaera apis* (chalkbrood) (vanEngelsdorp and Meixner 2010, Capri and Marchis 2013). Drought can increase neonicotinoid residuals in bee forage (Garavito 2017). Thus, disease and increased insecticide exposure due to inclement weather has the potential to further compromise bee health and reduces foraging capabilities.

Over a longer time-scale, climate change can reduce diversity, availability and quality of flowering plants, thus modifying foraging activities and causing deterioration of honey bee

nutrition (Scheper et al. 2014, Ziska et al. 2016, Mata 2018). Because global climate change increases temperatures and drier weather, plant diversity is declining, which can in turn endanger forage security for honey bees (Harrison et al. 2015). In conjunction with increasing CO_2 concentrations in the atmosphere and global warming, protein content in pollen has also been observed to decrease (Ziska et al. 2016). This may contribute to poorer honey bee nutrition, leading individual honey bees to have smaller body size and carry lighter forage loads during flight (Ziska et al. 2016). The Midwestern USA, where I conducted my dissertation work, is also subject to pronounced fluctuations in interannual weather and global climate change (Wuebbles et al. 2017), creating another factor concerning bee health in this challenging area for beekeeping.

4. Nutritional quality of forage affects foraging preference.

When individual honey bees forage for nectar or pollen, they are often faced with multiple different floral resources at one location. The nutritional quality of forage may be an important factor driving their foraging choice, i.e. on which flowers they concentrate their foraging efforts. Sugar and water are the main components of nectar, and sugar concentration is an important factor in honey bee foraging decisions. Honey bees show a preference for sugar concentration of 30 - 50 % (Waller 1972). Concentrations higher than this make viscosity too high so that the nectar is harder for honey bees to imbibe, while lower concentrations are unfavorable because of decreased net caloric returns for each foraging trip. However, preferences for specific sugar concentrations may change when the nutritional needs of colonies change (Hendriksma et al. 2019).

Other trace components in nectar such as amino acids and phytochemicals may also influence honey bees' foraging preferences. Sugar solutions added with amino acids are generally more preferred compared to sugar solution without amino acids (Inouye and Waller

1984, Kim and Smith 2000). Nectar amino acid composition can also affect honey bee foraging preference, and honey bees prefer to forage on nectar with more essential amino acids than non-essential amino acids (Hendriksma et al. 2014). Honey bee foraging preferences may also be affected by mineral concentrations, e.g. high levels of some minerals, such as potassium, are less preferred (Waller et al. 1972, Afik et al. 2008). Phytochemicals in nectar such as alkaloids (nicotine, caffeine and amygdalin) at low levels can increase foraging preference (Hagler and Buchmann 1993, London-Shafir et al. 2003, Singaravelan et al. 2005), while other phytochemicals such as phenolics in nectar can deter foraging (Hagler and Buchmann 1993).

Pollen is the only source of crude dietary protein for honey bees, which is then broken down into amino acids and absorbed in the midgut. Honey bees have a general preference for pollen with higher protein content (Levin and Bohart 1955, Schmidt and Johnson 1984, Waddington et al. 1998, Russo et al. 2019), however, they do not always choose pollen with higher protein content over lower protein content (Levin and Bohart 1955, Schmidt 1982), suggesting other pollen components such as lipids may also affect pollen preference. As availability of pollen resources can vary across seasons, forager bees may resort to collecting whatever is available to satisfy their nutritional needs, without a strong preference for pollen of high protein content and even accepting pollen of poor nutritional value (Moezel et al. 1987, Pernal and Currie 2001).

The composition of pollen protein, i.e. essential vs nonessential amino acids, may also affect honey bees' foraging preference. Honey bees prefer to forage on pollen with a greater portion of essential amino acids when given various pollen diet choices (Cook et al. 2003). Honey bees may also choose to forage on an array of diverse pollens to balance amino acid nutrition needs, e.g. when pollen from one plant complements the amino acid deficiency of

another (Hendriksma and Shafir 2016). In addition to deliberately collecting polyfloral pollen, monofloral pollen of high quality is also preferred by honey bees (Schmidt 1984); both polyfloral and high quality monofloral pollen can improve honey bee immunity to parasites and viruses (Di Pasquale et al. 2013, Dolezal et al. 2019b).

Lipids are also important components of pollen than can influence forage preferences. Lipid content may provide an important olfactory stimulus to honey bees, and pollen of higher lipid content is preferred by honey bees compared to that of low lipid content (Singh et al. 1999). Free fatty acids are an important component of pollen lipid content, and honey bees prefer pollen with more unsaturated fatty acids compared to pollen with less unsaturated fatty acids or more saturated fatty acids (Hopkins et al. 1969).

5. Variation in landscape composition affects availability and diversity of forage for honey bees.

Honey bee colonies are frequently kept by beekeepers in agricultural landscapes, both for pollination services and honey production. Cropland can provide forage resources for honey bees, including flowering crops such as sunflowers and canola, that can provide large quantity of forage to honey bees during the crop's blooming period (Requier et al. 2015, Thom et al. 2018). However, cropland forage habitats may have many potential negative impacts on honey bee health. First, pesticides such as insecticides, herbicides and fungicides are frequently applied to crops for suppressing insect pests and weed, and preventing crop diseases; such applications and their residues can expose honey bees to these toxic chemicals. In addition to insecticides poisoning honey bees, the negative effects of herbicides and fungicides have also been recognized recently (Johnson and Percel 2013, Balbuena et al. 2015, Motta et al. 2018). Second, many mass flowering crops such as canola that are attractive to honey bees can predominate their diet at the expense of a more diverse diet, which could reduce overall nutritional health. Third, mass crop bloom may provide a feast of a single food type to honey bees, but flowering duration

of many crops is short and honey bees can suffer from forage shortage after crop bloom. Thus, cropland may not be a reliable and high-quality forage source for honey bees (Dolezal et al. 2019a), which require sustained and diverse forage to satisfy their nutritional needs across the growing season.

In addition to cropland, urban land cover is another widespread landscape type that can be a source of forage for honey bees (Garbuzov et al. 2015). Compared to cropland, previous studies show inconsistent results on the value of urban land for honey bees, demonstrating both higher (Donkersley et al. 2014, Lecocq et al. 2015) and lower (Couvillon et al. 2014, Lecocq et al. 2015, Sponsler and Johnson 2015, Sponsler et al. 2017) forage availability. Regional variation in floral availability and quality in urban areas and croplands may be the cause of these mixed results. If gardens, roadsides, and parks in urban areas have diverse ornamental flowering plants (Garbuzov et al. 2015, Somme et al. 2016), they may be better forage sources than cropland. If floral resources are limited in urban areas, they may be inferior forage sources for honey bees compared to cropland, since crops and other floral resources adjacent to cropland like ditches and field margins can harbor both weedy and native floral resources such as clover (*Trifolium* spp.) and goldenrod (*Solidago* spp.) (Requier et al. 2015, Dolezal et al. 2019a).

Natural woodland habitat can also be a valuable forage source for honey bees (Hill and Webster 1995, Nagamitsu and Inoue 1999, Jung and Cho 2015, Mensah et al. 2017). When bee colonies are closer to woodlands containing flowering trees, they collect more forage (Sande et al. 2009). If honey bees are used for crop pollination, the presence of blooming forest trees may affect pollination efficiency by attracting honey bees away from flowering crops (Gaines-Day and Gratton 2016). Several tree taxa are common nectar and/or pollen sources for honey bees across the world, including maple, *Acer* spp. (Scullen and Vansell 1942, Haragsim 1977,

Ginsberg 1983, Batra 1985, Sanford 1988, Williams et al. 1993, Farkas and Zajácz 2007, Modvala et al. 2016), willow, *Salix* spp. (Scullen and Vansell 1942, Lieux 1975, Sanford 1988, Day et al. 1990, Farkas and Zajácz 2007, Kaškonienė et al. 2010, Modvala et al. 2016), chestnut, *Castanea* spp. (Williams et al. 1993, Vossen 2000, Tsigouri et al. 2004, Farkas and Zajácz 2007, Castro-Vazquez et al. 2010, Modvala et al. 2016), black locust, *Robinia pseudoacacia* (Scullen and Vansell 1942, Farkas and Zajácz 2007, Niculina et al. 2012, Jung and Cho 2015, Wojda et al. 2015, Carl et al. 2017) and basswood/lime/linden, *Tilia* spp. (Scullen and Vansell 1942, Farkas and Zajácz 2007, Wojda et al. 2015, Modvala et al. 2016). In temperate North America, maple, willow and basswood are important sources of forage for honey bees. It is important to note that the presence and flowering time of these key tree taxa can vary according to latitude and altitude (and all are found in Iowa, the location of this dissertation). However, woodland cover varies greatly depending on habitat type and region, and may not provide abundant forage to honey bees in periods when key forage trees cease blooming. Natural habitats other than woodlands, such as grasslands, may provide alternative natural habitats for forage if apiaries do not have access to woodlands, or if a woodland has limited key forage trees.

Nonnative grasslands such as pastures and rangelands managed for grazing can vary in their diversity of flowering forbs depending on location and management; flower-rich managed grasslands can be good sources of forage for honey bees (Gallant et al. 2014, Sanderson 2016, Bendel et al. 2019, Clarice et al. 2020). However, if a grassland is dominated by grasses and lacking in flowering forbs, it will be of low value as a forage source for honey bees (Smith 1964, Palmer 2008). Native grasslands, including both native tropical grasslands such as savannas (Abdullahi et al. 2011, Dukku 2013) and native temperate grassland such as steppe habitats extending across eastern Europe and central Asia (Kim 2018), pampas in South America

(Malkamäki et al. 2016) and prairies in North America (Nelson and Jay 1982) may provide favorable choices as apiary locations. Conservation grasslands such as CRP (Conservation Reserve Program) areas in the U.S. containing native prairie plants have been shown to support honey bee colonies (Otto et al. 2018).

As honey bees can fly several kilometers (up to 13.5 km) to collect forage in mixed landscapes composed of different habitat types such as cropland, urban, woodland and grassland, variation in landscape composition around honey bee hives can affect available forage (Beekman and Ratnieks 2000). In a landscape dominated with cropland, in addition to flowering crops honey bees also forage floral resources from natural habitats (Odoux et al. 2012, Danner et al. 2016). Honey bees in agricultural landscapes with more surrounding natural habitat collect more nectar compared to those with less natural habitat (Smart et al. 2016). However, higher natural habitat cover in crop dominated landscapes may not necessarily increase pollen diversity and abundance collected by honey bees (Danner et al. 2017). The lack of enhancement in pollen forage may be due to not enough natural habitat being present, or lack of preferred flowering plants in the natural habitats used by honey bees for forage. Alternatively, there may be flowering plants attractive to honey bees in patches of natural habitat, but the abundance of those species is insufficient.

6. Extensive corn and soybean agriculture affects honey bee health.

In the Midwestern USA, cropland cultivated with soybean and corn is the dominant land cover. Iowa, the state in which my dissertation work was conducted, has 64 % of land cover in annual crops (USDA-NASS 2019). This region has been identified as a critical area for pollinator health (Grixti et al. 2009, Zaya et al. 2017), and annual colony losses in Iowa are extremely high, with an average 60.15 % total annual losses, ranging between 34.9 - 76.4 % as surveyed within five years during 2010-2011 and 2012-2016 (vanEngelsdorp et al. 2012,

Steinhauer et al. 2014, Lee et al. 2015, Seitz et al. 2015, Kulhanek et al. 2017). These colony losses are appreciably higher than nationwide means and greatly above historically sustainable loss rates of 15%. High losses in the upper Midwestern U.S. have been mainly attributed to stressors associated with extensive agricultural land conversion (namely habitat loss and pesticides), as well as the harsh and challenging climate of this region (Wuebbles et al. 2017).

Despite predictions that honey bees in monoculture dominated landscapes should suffer from health deficits, a previous study that I co-authored found that Iowa colonies located in high cultivation (high percentage of cropland) landscapes are heavier in contrast with those with low cultivation landscapes (low percentage of cropland) (Dolezal et al. 2019a). We found evidence that flowering soybean and weedy flowers such as several species of clover (*Trifolium pretense* and *Trifolium repens*) growing along the country roads and lawns adjacent to cropland likely contribute to the heavier colony weight in low cultivation landscapes. However, after crops and clover cease blooming, colonies in Iowa lose weight no matter where the colonies are located, suggesting that cropland is a transient forage resource for honey bees and other land covers lack floral resources in the late season (Dolezal et al. 2019a). However, when colonies are moved to native prairie habitats after crop blooming, colonies regain weight, likely because of the rich floral resources found during the late season in prairies. In my dissertation, I further investigated which native plants in prairies are used as honey bee forage, and addressed whether prairies consistently provide pollen to honey bees across the season.

Previous studies suggested, but did not definitively show, that honey bees consistently use prairie plants as forage, based on studies analyzing honey bee foraging recruitment communication (the famous “waggle dance”) (Tuell et al. 2008, Carr-Markell et al. 2020). The Midwest was historically covered by a high portion of land as prairie prior to European

settlement, up to 85 % of Iowa land was at one time covered by tallgrass prairie (Smith 1981). However, at the current time the vast majority of prairie (82.6 - 99.9 %) has been replaced by cropland and urban land in the Midwest (Samson and Knopf 1994) and Iowa is one of states with highest prairie losses (99.9 %). In recent years, there has been interest in restoring prairies in these regions while preserving Midwesterners' agricultural way of life. One conservation practice, Science-Based Trials of Rowcrops Integrated with Prairie Strips (S.T.R.I.P.S. Project), launched by Iowa State University (ISU) is bringing native prairie vegetation back to cropland to improve farming quality and biodiversity by only taking a small portion (usually < 10 %) of crop field into prairie patches (Schulte et al. 2017). My dissertation research therefore assessed whether prairie strips can be a reliable source of forage source for managed honey bees and support healthy colonies.

Dissertation Objectives

The aims of my dissertation are listed as follows:

1) Determine if low cultivation (low percentage of cropland) landscapes provide more abundant and diverse pollen forage to honey bees than high cultivation landscapes (high percentage of cropland), and if the most common pollens collected in agricultural landscapes are of sufficient quality to buffer honey bees' response to virus infection.

2) Investigate if land cover types in the Midwestern U.S. and/or annual weather fluctuations affect abundance and diversity for pollen collected by managed honey bee colonies.

3) Determine which plants in tallgrass prairies are used by honey bees for pollen forage, and examine the seasonal dynamics of this forage, i.e. examine whether prairies can be a continuous source of pollen forage for honey bees throughout the growing season.

4) Evaluate if the prairie strips conservation approach can provide managed apiaries with more abundant and diverse pollen forage and lead to larger, healthier colonies as evidenced by

enhanced colony weight (more food stores and wax produced) and larger immature and mature bee populations.

Dissertation Organization

This dissertation summarizes my work studying how agricultural landscapes, various land uses, native prairie habitats and conservation habitats (prairie strips) affect abundance and diversity of forage that determine colony growth and health. **Chapter 1** is an introduction on honey bee nutritional requirements, abiotic and biotic factors affecting forage collection by honey bees, and previous knowledge on landscape effects on the abundance and diversity of forage. **Chapter 2** is entitled "Honey bee (*Apis mellifera*, Hymenoptera: Apidae) pollen forage in a highly cultivated agroecosystem: Limited diet diversity and its relationship to virus resistance", and has been published in Journal of Economic Entomology. In this chapter, I study if honey bee colonies maintained in areas of lower corn and soybean production in central Iowa can provide more abundant and diverse pollen to honey bees. Furthermore, I investigate the nutritional value of the most commonly collected pollens from these landscapes with respect to their value in supporting honey bees' resistance to viral infection. **Chapter 3** is entitled "Variation in annual weather, rather than land use, affects honey bee pollen collection in an agricultural landscape" and investigates if diverse vegetable and fruit farms and native prairies can provide more pollen forage compared to soybean farms. As a multi-year study, I also examined correlations with annual weather fluctuations and found effects on the abundance and diversity of pollen collected honey bees in this agricultural landscape. **Chapter 4** is entitled "North American prairie is a source of pollen for honey bees" and determined which plants in Iowa's reconstructed prairies are used by honey bees. This chapter also determine how pollen abundance and diversity collected by honey bees varied throughout the growing season. In addition to prairie-derived forage, I also document that honey bees utilize pollen forage from

other parts of the landscape, even when they are placed in and have access to high quality prairie habitat. **Chapter 5** is entitled "Prairie strips improve biodiversity, and honey bee forage and health in agricultural landscapes" and evaluates if prairie strips are an effective way to improve forage availability and colony growth by comparing apiaries located in prairie strips embedded in crop fields to those located at crop fields without prairie strips. **Chapter 6** is a summary of findings in the previous Chapters 2-5.

Overall, this dissertation aims to improve our understanding of how honey bee nutritional health is related to agricultural and native foraging habitat. I hope findings in my dissertation will make improvements in beekeeping in the Midwestern USA as well as other regions throughout the world.

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CHAPTER 2. HONEY BEE (*APIS MELLIFERA*, HYMENOPTERA: APIDAE) POLLEN FORAGE IN A HIGHLY CULTIVATED AGROECOSYSTEM: LIMITED DIET DIVERSITY AND ITS RELATIONSHIP TO VIRUS RESISTANCE

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M.O., A.T., A.D. and G.Z. conceived the ideas and designed methodology; G.Z., A.S. and A.D. collected the data; G.Z. analyzed data; G.Z. led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Abstract

Intensified agriculture reduces natural and semi-natural habitats and plant diversity, reducing forage available to honey bees. In agricultural landscapes of Iowa, USA, we studied the impact of extrinsic agricultural intensification on the availability of pollen for honey bees by placing colonies next to soybean fields surrounded by either a low or high level of cultivation. The abundance and diversity of pollen returned to a colony was estimated by placing pollen traps on bee colonies during the summer and fall of 2015 and 2016. We observed no difference in

abundance and diversity of pollen collected by colonies in either landscape, but abundance varied over time with significantly less collected in September. We explored if the most commonly collected pollen from these landscapes had the capacity to support honey bee immune health by testing if diets consisting of these pollens improved bee resistance to a viral infection. Compared to bees denied pollen, a mixture of pollen from the two most common plant taxa (*Trifolium* spp. and *Chamaechrista fasciculata*) significantly reduced honey bee mortality induced by viral infection. These data suggest that a community of a few common plants were favored by honey bees, and when available, could be valuable for reducing mortality from a viral infection. Our data suggest a late season shortage of pollen may be ameliorated by additions of fall flowering plants, like goldenrod (*Solidago* spp.) and sunflower (*Helianthus*, *Heliopsis & Silphium* spp.), as options for enhancing pollen availability and quality for honey bees in agricultural landscapes.

Keywords

Soybean, legume, virus, honey bee, *Apis mellifera*

Introduction

Honey bees in the USA and Europe are exposed to multiple environmental stressors (vanEngelsdorp et al. 2012, Goulson et al. 2015), including a reduction in forage quantity and diversity. Intensified agriculture dramatically reduces the availability of natural and semi-natural habitats, and is predicted to affect availability and diversity of forage across the entire growing season by decreasing overall plant diversity (Naug 2009). Pollen is a critical source of nutrition as it supplies proteins, lipids, and micronutrients (Wright et al. 2018). In agricultural landscapes, some mass flowering crops, like sunflower and oilseed rape, can provide honey bees large quantities of pollen in a short period (Requier et al. 2015, Thom et al. 2018). However, a preponderance of only a single species could contribute to reduced nutritional value of pollen for

honey bees (Schmidt 1984, Schmidt et al. 1987, Schmidt et al. 1995, Di Pasquale et al. 2013, Nicolson and Human 2013). After a crop blooms, honey bees may suffer from a shortage of forage in the surrounding landscapes due to limited natural or semi-natural habitats.

The value of crop and non-crop habitat as a source of pollen forage for honey bees can be important to inform land and apiary management decisions. In previous studies, enhancements in pollen collection were not detected when honey bee colonies were placed in landscapes with varying amounts of non-crop habitats (Danner et al. 2017). A general conclusion from these studies is that landscapes with less crop production do not necessarily result in an increase in the abundance and diversity of pollen collected by honey bees. One reason may be that these studies were performed in agricultural landscapes with a limited range of landscape diversity, resulting in reduced power to detect the effect of non-crop habitats on pollen forage (Danner et al. 2017). Another potential explanation may be the impact of the primary crop within these landscapes. Primary crops that are a source of pollen for honey bees may distract them from forage available in non-crop habitats (Danner et al. 2017). The response of honey bees to non-crop habitat may be stronger in landscapes where the crops are not a source of high-quality pollen for honey bees, such as soybean and corn. Corn pollen is considered to be of limited nutritional value for honey bees (Höcherl et al. 2012) and less likely to be a collected by honey bees. Honey bees can be found in soybean fields (Gill and O'Neal 2015) and collect nectar from soybean flowers (Villanueva-Gutiérrez et al. 2014, Wheelock et al. 2016, USDA 2017). However, soybean neither requires insect pollination, nor is it reported as a major pollen source for honey bees. Honey bees in a landscape dominated by these crops may focus more of their pollen foraging efforts in non-crop areas of a landscape rather than cultivated areas.

In this study, we predicted that honey bees kept in a landscape of low forage diversity and quality (i.e., where corn and soybean are extensively produced) would collect less abundant and a less diverse mixture of pollen, than those kept within a landscape with more non-crop habitat. We focused our study in the U.S. state of Iowa, where around 85% of the land is devoted to agriculture, and 64% of that land is used for corn and soybean production (USDA-NASS 2019). The intensive management of weeds in the Midwest has reduced floral diversity (Otto et al. 2016). To maximize our potential to observe honey bees using non-crop forage within this region, we selected soybean fields in locations that represented extremes of land use (i.e., landscapes that varied in the amount of farm cultivation as defined in Dolezal at al. (2019a). We predicted that honey bees kept in a landscape with a low amount of cultivation (i.e., corn and soybean production) would collect a greater quantity and diversity of pollen than those in a landscape with a high amount of cultivation. This prediction is based on an assumption that honey bees benefit and use forage available in non-cropped features of the Iowa landscape (woodland, old fields and pastures, and semi-urban to urban areas).

We also sought to understand the value of the most commonly collected pollen in this landscape, by focusing on the capacity of the pollen diet to support honey bee immune health. In addition to declining forage, pathogens, especially viruses, are considered a significant source of mortality for honey bees (Grozinger and Flenniken 2019). Augmenting the amount and type of pollen can improve honey bee immunity to pathogens (Parrinello et al. 2011, Foley et al. 2012, Di Pasquale et al. 2013). When provided a diet composed of pollen from diverse plants or from a single plant that produces high-quality pollen, honey bee survival was improved when infected with *Nosema* parasite and lethal viruses (Di Pasquale et al. 2013, Dolezal et al. 2019b). However, a diet of pollen from a single plant species of low-nutritional quality did not rescue bees from

Nosema infection (Di Pasquale et al. 2013). Monofloral pollen of low quality reduced honey bee mortality when infected with virus compared to bees denied pollen, but this mortality was higher than honey bees fed a ploy floral mixture of pollen (Dolezal et al. 2019b). However, the plants that were the source of pollen used in these studies are not relevant to Midwestern agricultural system. We determined the most commonly collected pollen by honey bees in central Iowa. This information was used to determine which plant species to include in an assessment of the value of pollen collected in central Iowa for protection from viral infection. We predicted that the differences in pollen diet found in central Iowa would affect honey bee resistance to virus infection.

Materials and Methods

Measuring the impact of land use on the diversity and abundance of pollen collected by honey bees

Study Sites

We summarize pollen foraging data from honey bee colonies that were part of a larger study exploring the impact of crop production on honey bee health (Dolezal et al. 2019a). This study demonstrated that landscapes surrounding apiaries of four colonies affected components of honey bee health (colony weight, adult and pupa populations, lipid concentration of individual nurse honey bees). Below we briefly summarize how the locations were selected.

The colonies deployed in this study were kept in a three-county region of central Iowa, USA. To control for variation immediately adjacent to our honey bee colonies, we placed apiaries next to commercial soybean fields. In 2015, we selected 10 soybean fields in Boone, Marshall and Story Counties. Because soybeans are rotated yearly in central Iowa, locations changed between 2015 and 2016, resulting in a different set of 10 soybean fields in Boone and Story Counties in 2016 (Fig. 1). To test our prediction that land use around a honey bee colony

affected pollen foraging, we looked for soybean fields that were surrounded by a landscape that fell within two categories: low and high cultivation. We defined cultivation as the amount of corn and soybean grown within 1.6 km radius of the field edge where the honey bee colonies would be located. The percentage of these two crops that occupied the buffers around the colonies were calculated based on the amount of other land uses considered 'non-crop' (i.e., woodland, urban, pasture and prairie). The amount of each land use type for a location was measured with ArcMap (Esri, Redlland, CA) from data collected by USDA-NASS (https://nassgeodata.gmu.edu/CropScape/). Details regarding the classification of non-crop area and specific details for each location can be found in the larger study (Dolezal et al. 2019a).

The average amount of non-crop habitat around fields within each category varied by year. For fields considered in the high cultivation category, the average amount of non-crop habitats was 18.4% and 13.6% in 2015 and 2016, respectively (Table 1). For fields in the low cultivation category, the average amount of non-crop habitat was 54.2% and 69.2% in 2015 and 2016, respectively. Previous studies have demonstrated that this low versus high cultivation classification scheme resulted in different communities of insects within a focal soybean field as well as differing nectar dynamics for honey bees (Gardiner et al. 2009, Bennett and Isaacs 2014, Dolezal et al. 2019a). By using these two extreme categories, we predicted that land use differences would provide significantly different amounts and diversity of flowering resources for our apiaries.

An apiary of four colonies was placed at the field edge of each soybean field (5 per landscape category per year) that was managed using conventional practices with regards to pesticides (fungicides and herbicides), fertilizer and tillage use. Because honey bees have been estimated to mainly forage (around 90% visitation) for pollen within a 1.6 km radius of their

colonies in agricultural landscapes (Couvillon et al. 2014, Danner et al. 2014), we selected fields such that any two apiaries were at least 3.2 km apart from each other. In this way, we attempted to limit overlapping honey bee foraging ranges between fields of different landscapes so that the pollen collected from each apiary could be considered independent. This distance resulted in average colony weight varying significantly between apiaries kept at soybean fields in low vs high cultivation landscapes (Dolezal et al. 2019a).

Honey bee apiaries

An apiary of four colonies housed in Langstroth hives was placed together on a wooden pallet at the field edge of each soybean field. These colonies were part of the larger experiment and a more detailed response of colonies to the two landscape categories is described in terms of differences in colony weight, brood amount, adult bee population (Dolezal et al. 2019a). To reduce the potential negative effect of pollen traps on colony health, only one of the four colonies at each field was used to measure pollen collection, but the management was the same for all colonies within an apiary.

Apiaries were first established at an Iowa State University (ISU) farm and then distributed to our study fields on 10 June 2015 and 23 May 2016. In 2015, the colonies were started from 0.9 kg packages of bees (about 7,000 bees); while in 2016, colonies were started from nucleus colonies consisting of approximately three frames of worker bees (similar amount to 2015). To reduce variation due to genetic lineage, queens used in all colonies in both years were *Apis mellifera ligustica*. Each colony was inspected once every 14 d as part of a standard protocol to measure colony health, including checking for the presence of the queen. If we did not observe the queen or sign of her activities (i.e. egg or young larvae), the colony was re-queened within 1-2 days with a queen from the same commercial source from which colonies

were derived. Colonies were not fed supplementary feed throughout the experiment. Other apiary management details were demonstrated in another journal publication (Dolezal et al. 2019a).

Pollen collection

One colony in each apiary was randomly selected to receive a pollen trap attached to the colony entrance (Brushy Mountain Bee Supply, Wilsonville, U.S.). Foraging honey bees must enter the trap to return to the colony entrance, and a plastic plate with many star-shaped holes in the trap pulls pollen pellets from corbiculae of individual bees. Dislodged pollen pellets fall into a basket under the trap. When not in use, the plastic plate was removed, allowing foragers to return undisturbed into the colony.

A total of ten pollen collections were taken from July to September in 2015, and 13 from June to September in 2016. Those pollen collections resulted into 100 pollen samples in 2015 and 130 samples in 2016. Pollen traps were opened for 24 h on each collection without rain. After the non-pollen debris was removed, pollen samples were weighed and stored at -20 °C for later taxonomic identification.

Pollen identification

We used a compound light microscope to view morphological features of the pollen grains to identify from which plant species they were collected. A 2 g subsample of pollen collected at each field and date was first sorted according to color. Pollen of different colors was weighed and dissolved in Caberla's solution using fuschin dye and then mounted onto glass slides. To identify pollen to the lowest taxonomic level, pollen from the traps was compared to pollen extracted from flowers obtained from the study areas during the period when pollen traps were open. Pollen types that were not identified based on this reference collection were recorded as unknown and given a separate morphospecies designation. Pollen diversity was assessed by

species richness (number of plant taxa represented by the pollen) and the species richness and evenness of plant taxa represented in a collection was assessed using Shannon's diversity index.

Measuring the effect of variation in pollen diet on honey bee immune-health

To test if pollen from the most commonly collected species within the central Iowa landscape affected honey bees' resistance to viral infection, we conducted a laboratory-based experiment on the campus of ISU in 2017. There were two experimental factors: virus infection (two levels; present or absent) and diet source (four levels; described below), accounting for eight treatments, with 24 replicates per treatment for a total of 192 experimental units. Each experimental unit consisted of a cage containing 30 newly emerged honey bees.

The no-pollen diet served as a negative control and chestnut pollen (*Castanea* spp., purchased from Pollenergie, Saint-Hilaire-de-Lusignan, France) as a positive control. Chestnut pollen was selected as it has high levels of protein and antioxidants, and in a similar assay was observed to rescue honey bees from a lethal dose of the microsporidian pathogen *Nosema ceranae* (Di Pasquale et al. 2013) and a mixture of viruses (Dolezal et al. 2019b). The other two pollen diets were based on the most commonly collected pollen from our field experiment: clover pollen (*Trifolium* spp.) and a 50% : 50% mixture of clover and partridge pea (*Chamaecrista fasciculata*) pollen. Clover pollen consisted of approximately 50% red clover (*T. pratense*) and 50% white clover (*T. repens*) pollen. The pollen diets were fed to bees by mixing three portions of each pollen group with one portion of 50% sucrose water free of virus.

The virus inoculum was produced according to methods used by (Carrillo-Tripp et al. 2016, Dolezal et al. 2019b). Five common virus types were screened in our inoculum, including acute bee paralysis virus (ABPV), black queen cell virus (BQCV), deformed wing virus (DWV), Israeli acute paralysis virus (IAPV), Kashmir bee virus (KBV) and sacbrood virus (SBV). Of these, only IAPV, BQCV and SBV were detected as follows: 85% IPV, 14% SBV and 1%

BQCV. Primers used for identification and quantification of virus compositions were the same as those used in the two previous studies. Virus inoculum in phosphate-buffered saline (PBS) was diluted with 30% sucrose solution by 1:750 ratio prior to the feeding. Caged bees were infected by consuming the 600 µl sucrose solution containing virus inoculum. Our no-virus infection controls were fed a sterile sucrose solution identical to that used in the treatment except with 0.8 µl sterile PBS containing virus particles. Previous work has shown that, using these procedures, IAPV is the primary replicating virus, including when SBV is present in even higher quantities, and is the cause of honey bee mortality (Carrillo-Tripp et al. 2016, Dolezal et al. 2019b).

Honey bees used in this experiment were collected from brood frames identified with emerging bees from five colonies at the ISU apiary. All adult bees were removed from these frames before they were brought to a laboratory on the campus of ISU. These capped brood frames were kept in wooden boxes within a rearing room (33°C and 80% relative humidity). After 24 h, newly emerged bees were collected, mixed and randomly assigned to 192 acrylic cages (eight treatments comprised of 24 cages per treatment) with 30 bees per cage (10.6 × 10.16 × 7.62 cm).

The four pollen diets were randomly assigned to each cage with newly emerged honey bees, with 2.2 g pollen placed at the bottom of each cage. The pollen diets were renewed every 24h. Half of all replicates (96 cages) were randomly assigned exposure to the virus mixture. A volume of 600 µl diluted virus solution was provided to honey at a plastic bowl on bottom of the cage bees immediately after feeding pollen diets for the first time. The remaining 96 cages were provided with 30 % sucrose solution without virus. Once the virus solution was consumed, all bees were fed *ad libitum* with a virus-free 50 % sucrose solution through 15 ml plastic tubes on

the top of the cage during the rest of the experimental period. Dead bees were removed every 24 h and the amount recorded to calculate % mortality.

Statistical analysis

To determine the effect of varying landscapes on the abundance and diversity of pollen forage to honey bees, we used a repeated measure design by monitoring pollen collection throughout the growing season. Linear mixed models were used to conduct an analysis of variance (PROC MIXED) on pollen amount and diversity using SAS 9.3 software (SAS Institute, Cary NC). Pollen amount (g) and diversity (species richness and Shannon diversity) was the dependent variable, with landscape as the independent variable, date as the repeated variable, and field as a random effect in the analysis. We used an AR1 (autoregressive) structure (PROC MIXED) for the correlation of amount of pollen or diversity collected among different dates to obtain the lowest AIC and AICC value in the above model. Honey bee colony population grows over time and, to reduce the variation of colony size on foraging behavior, we standardized the amount of pollen collected by colony weight, which included the mass of adult bees, brood, honey and pollen. To improve the normality of the pollen abundance data, the amount of pollen was transformed (base-10 log) prior to analysis when necessary. Due to the difference between colony arrival dates to the fields and starting colony size, the amount of pollen collected was analyzed separately for both years. Pollen amount and diversity from the two landscape categories at each date was also compared using least square means under the condition of a linear mixed effects model.

To describe the patterns of pollen collected over time, the average amount of pollen of different months (normalized by average colony mass of each month) was compared using analysis of variance (PROC MIXED) and Tukey-Kramer HSD multiple comparisons. To

demonstrate how major pollen types changed over time, we organized the source plants of pollen collection into three groups: clover, partridge pea and trace pollen. Because white clover and red clover are from the same genus and have similar blooming periods in central Iowa, we included them into one group. Pollen from plants that represented < 5% of all pollen collected was grouped as trace pollen.

The cage experiment was conducted as a fully crossed, completely randomized design, with every combination of virus and diet treatment represented in the analysis. To test the efficacy of the virus treatment, mortality of virus-treated honey bees was compared with that of untreated honey bees using a Welch's two sample *t*-test (PROC TTEST). To determine if different pollen diets affected honey bee survival when challenged by the virus, the % mortality of honey bees fed by different diets was analyzed within virus-treated bees by analysis of variance (PROC MIXED).

Results

Pollen abundance in apiaries within differing landscapes

The amount of pollen collected by honey bees throughout the sampling period did not differ between colonies located in either the low or high cultivation categories in 2015 (F = 0.36; df = 1, 10.3; $P = 0.728$; Table 2, Fig. 2A) or 2016 (F = -0.80; df = 1, 15.10; $P = 0.437$; Table 2, Fig. 2B). On one specific date, 18 August 2016, the amount of pollen collected in the high cultivation landscapes was significantly greater than that from the low cultivation landscapes (Fig. 2B, $t = -2.18$; df = 49.7; $P = 0.034$). Otherwise, there were no significant differences in the amount of pollen collected between the two landscape categories on any other date in both 2015 and 2016.

Diversity of plant species used for pollen

In 2015, both landscapes categories had 25 plant taxa found in our collection (Table 3, Supp. Tables 1 & 3). Among plant taxa identified in our pollen, 11 taxa were shared between the two landscape categories; among pollen from unidentified plant taxa, only four were shared between the two landscape categories (Supp. Table 3). Six native plant taxa were used by honey bees with four of these shared between two landscape categories. *Ratibida pinnata* and *Phlox paniculata* were collected only by colonies in the low cultivation categories (Table 3).

In 2015 clover was the most abundant pollen by mass, while partridge pea was the other abundant pollen collected by honey bees (Table 3, Supp. Figs. 1-2). Over 90% of the total pollen brought back to the colonies throughout the entire experimental period was comprised of clover (*Trifolium* spp.) and partridge pea (*Chamaechrista fasciculata*) in both landscape categories (Table 3). Among 100 pollen samples collected on different days during 2015, 50 ones were composed of > 90 % clover.

In 2016 a total of 51 and 54 plant taxa found in bee-collected pollen were from high and low cultivation landscape, respectively (Table 4, Supp. Tables 2-3). A total of 21 plant taxa identified in pollen were shared between two landscape categories, so did 20 plant taxa unidentified in pollen (Supp. Table 3). Ten native plant taxa were used by honey bees with eight of these shared between two landscape categories (Table 4, Supp. Table 3). The native plants *Zizia aurea* and *R. pinnata* were collected only by colonies in the high cultivation landscape (Table 4). Clover (*Trifolium* spp.) and partridge pea accounted for over 73% of the total pollen collected in the entire experimental period identified in both landscapes during 2016 (Table 4). A number of 39 out 130 pollen samples were composed of > 90 % clover.

Taxa richness and Shannon diversity of pollen brought to colonies did not significantly differ between landscape categories in both years ($P > 0.05$) (Table 2). Across individual dates in

both years, we did not observe any difference in the richness and Shannon diversity of pollen between low vs high cultivation landscapes ($P > 0.05$ for all dates) (Figs. 3-4). Taxa richness was generally in the range of 2-6 taxa per 24 h sampling period in 2015 and 2016 (Fig. 3). Shannon diversity index had similar patterns as richness (Fig. 4).

In summary, the diversity of plants used by bees in the two landscape categories were very similar. In 2015, the were no differences in the plants used and in 2016, only two more taxa were found in the pollen of colonies kept in the low cultivation landscape. Therefore, we conclude that locating bee colonies in low cultivation landscapes did not increase the number of plants taxa used by honey bees for pollen forage. And a larger portion of plant taxa foraged by honey bees were shared between the two landscapes. Among the plant taxa that were identified in the pollen, all the native plants can be found in grasslands and prairies (Tables 3 and 4, Supp. Table 3), except *Tilia americana* which is a woodland species. The nonnative plant taxa were most likely found in agricultural components of landscape (Tables 3 and 4, Supp. Table 3).

Despite being next to soybean fields, we did not observe any soybean pollen in the pollen traps during both years. However, other legumes, clover and partridge pea, were the most commonly collected pollen in both years (Tables 3 & 4). Corn was rarely a source of pollen (< 1 % in 2015 and < 2 % in 2016) though corn was very abundant.

Phenology of pollen

Although we did not detect a difference in the amount of pollen collected by honey bees kept in the low versus high cultivation categories, we did observe differences in the amount of pollen collected by sampling date (Table 2). When the amount of pollen was organized into three general groups (i.e., clover, partridge pea and trace pollen) and binned by month, we observed a remarkable decline in the amount of pollen returned to the colonies (Fig. 5). In both 2015 and 2016, honey bees collected the least amount of pollen by weight during September (Fig. 5). The

amount of pollen collected in September were only 12 % and 46 % of its peak weight in 2015 and 2016, respectively.

Variation in pollen diets affects honey bee immune-health

Using a method that has been shown to reveal variation in honey bee mortality to viral infection based on diets (Carrillo-Tripp et al. 2016, Dolezal et al. 2019b), we found honey bees receiving the virus treatment suffered significantly higher mortality than those untreated (t = 15.39; df = 109.48; $P < 0.0001$) (Fig. 6). In the absence of a viral infection, there was no significant difference in percent mortality among the pollen diets, including the no-pollen diet (F = 0.42; df = 3, 95; $P = 0.740$, multiple comparison by Tukey-Kramer HSD). However, for honey bees receiving the virus treatment, we observed a significant difference in mortality between infected honey bees provided the various pollen diets (F = 3.62; df = 3, 95; $P = 0.016$). Our positive control (i.e. *Castanea* pollen) confirmed that our assay could detect improvements in honey bee survival consistent with previously published studies (Di Pasquale et al. 2013, Dolezal et al. 2019b). Clover (*Trifolium* spp.) pollen alone did not significantly reduce honey bee mortality compared to the no-pollen diet, however the mixture of clover and partridge pea pollen significantly reduced mortality by 10% compared to the no-pollen diet (F = 3.62; df = 3, 95; P = 0.016, multiple comparison by Tukey-Kramer HSD).

Discussion

Equal abundance and diversity of pollen collection between two landscape categories

Our initial prediction that landscape variation around honey bee colonies kept adjacent to soybean fields would affect the amount and diversity of pollen collected by foraging honey bees was not confirmed. We predicted that both the abundance and diversity of pollen would be greater in colonies kept in the landscapes with low cultivation, as these would have a greater diversity of plants beyond corn and soybean. As anticipated, honey bees did not use either corn

or soybean as a significant source of pollen (Tables 2-3), indicating that these crops did not directly influence the pollen foraging behavior of honey bees. Contrary to our prediction, the abundance and diversity of pollen collected by honey bees did not vary between low and high cultivation landscapes even though fields within the low cultivation landscape contained more non-crop habitats than fields within the high cultivation landscapes.

These results were similar to previous studies in which the amount and diversity of pollen collected by honey bees was measured across colonies kept in multiple locations that varied in the diversity of land use surrounding honey bee colonies (Smart et al. 2016a, Smart et al. 2016b, Danner et al. 2017). There could be several explanations for why variation in land use did not affect the amount and diversity of pollen collected by honey bees in these studies. The simplest explanation may be that the occurrence of plants that represent the most commonly collected pollen (clover species, *Trifollium repens and T. pretense,* and partridge pea, *Chamaecrista fasciculata*) did not differ between the two landscape categories in our study. These two plant taxa are not intentionally planted in either landscape category in our study. The first most commonly collected pollen (clover species) came from plant species (clover) that are well-known sources of forage for honey bees (Sponsler and Johnson 2015). Although these clover species are not native to North America, it is common throughout the Midwest, in part because was intentionally added to pastures for livestock production. However, land committed to pasture currently makes up a small portion of the central Iowa landscape (Dolezal et al. 2019a) and clover is widely distribute in roadside, field margins and lawns as a weedy species (Turkington and Burdon 1983, Sponsler and Johnson 2015). These locations were components of both the low and high cultivation landscapes in Iowa where flowering clovers were found (Zhang pers. Observation). Clovers have a long blooming period from summer to early fall (Turkington and

Burdon 1983, Larson et al. 2014) that could be a source of honey bee forage. By the end of August, clover ceased to bloom and honey bee colonies begin to lose weight comprised mostly of honey (Dolezal et al. 2019a). Interestingly, the second most commonly collected pollen came from partridge pea (*Chamaecrista fasciculata*) after clover bloom. It is a native North America annual plant whose native habitat (prairie) has been reduced to less than .01% of its original range. Other land uses such as roadside, river banks and conservation land that are component of both landscape category were potential habitats for partridge pea used for versatile purposes such as cover crops for erosion control and improving soil fertility, forage for wild life and recreation (Hardin et al. 1972, Kauffeld 1980, Mannouris and Byers 2013, Houck and Row 2019). This is remarkable as honey bees are not native to Iowa but seem to prefer these rare sources of forage over more abundant sources of pollen, such as corn and soybean. Habitat that could potentially contain both clover and partridge pea represent a large amount of the area in both landscape categories, so estimating the floral population of these two plants is beyond the resources available to us at the time of this study. Clover pollen was found in all pollen traps during both years and partridge pea found in the majority of traps (at eight fields during 2015 and seven fields during 2016), suggesting that clover and partridge pea plants may be ubiquitous in central Iowa.

Phenology of pollen availability

Because honey bees forage throughout the growing season, there is a need to explore the response to landscapes over a phenological period that extends beyond the flowering period of the dominant crop(s) or non-crop sources of forage. We observed variation in pollen abundance over the season. As the amount of clover collected in colonies declined in August, partridge pea became more of the total pollen brought to colonies (Fig. 5, Supp. Figs. 1 & 2). The reduction in clover pollen occurred as clover ceased blooming (Dolezal et al. 2019a), and likely not due to a

greater attractiveness of partridge pea. Honey bees may have turned to partridge pea for enough forage leading to similar overall amount of pollen from both plants collected within the two landscape categories. Previous studies in Europe have identified August as a period of pollen dearth in temperate regions (Garbuzov et al. 2015, Requier et al. 2015, Danner et al. 2017), and this food shortage is considered to contribute to colony losses (Requier et al. 2017). Partridge pea blooming in August within central Iowa is an alternative source of pollen not readily available during the same time period in Europe.

By September, we observed a significant reduction in pollen brought back to the colonies. In September of both years, clover was still part of this collection, partridge pea was not found and a mixture of other plants became a source of pollen. Identifying a period of pollen shortage provides valuable information for aiding bee nutritional health by indicating when there is a need for alternative forage. Lower pollen availability in September may be critical for honey bees preparing for over-wintering (Fig. 5). Some native *Solidago* spp. and *Helianthus* spp. were sources of pollen for bees later in the growing season (Tables. 2-3). These species typically bloom during August and September and could help counter a shortage of pollen during September if seeded or planted near apiaries (Ginsberg 1983, Smart et al. 2016b, Wood et al. 2018). Future studies could focus on determining the value of plants that bloom in the later part of the growing season (e.g. August, September) when bee colonies rear new bees for overwintering that is critical for general colony health and overwinter survivorship.

Enhanced resistance to viral infection: a potential benefit from a diet of two pollen sources

Regarding our second prediction that varying pollen diet affect honey bee health, we selected pollens observed in our field study to determine their contribution to an aspect of colony health, i.e., resistance to virus infection. We compared a pollen diet composed of a mixture of pollen from two clover species (*Trifolium* spp.) to a mix of clover and partridge pea in

proportions similar to what we observed in the field. Clover pollen alone did not significantly reduce honey bee mortality from a viral infection compared to no pollen diet. When honey bees were provided pollen from both clover and partridge pea, mortality was significantly reduced compared to a no pollen diet. These results suggested that partridge pea may be more than just an alternative source of pollen late in the season but also an improvement in the quality of the honey bee diet. Given that pathogens and forage availability are considered key stressors experienced by honey bee and multiple species of wild bees, these data were interesting as they suggested an explanation for why honey bees are using a native plant as a source of pollen. These data also suggested that by conserving habitat that contains this native plant (and others potential sources of pollen after clover ceases to bloom), honey bees may be relieved of these stressors. Honey bees collected pollen from several other plant species throughout the course of this study (i.e., trace pollens), though in much lower quantities than either clover or partridge pea. To what extent these trace pollens could sufficiently improve the survival of honey bee's resistance to viral infection needs further exploration.

Value of legumes for honey bee pollen

Remarkably, several of the plants used by honey bees as a forage in central Iowa are legumes. Six legumes species were common sources of pollen regardless of where colonies were located, including nonnative white clover (*Trifolium repens*), red clover (*Trifolium pratense*), sweet clover (*Melilotus* spp.), birdsfoot trefoil (*Lotus corniculatus*), and native partridge pea (*Chamaecrista fasciculata*) and purple prairie clover (*Dalea purpurea*). Combined, these plants represented 93% or 81% of the total pollen collected by honey bees in two years of our study (Tables 3-4, Supp. Fig. 3). Although colonies were placed adjacent to fields of soybean, also a legume, we did not observe soybean pollen in any of the colonies. Previous studies have also demonstrated that at least one of those legumes found in our study was a major source of pollen

for honey bees in other states of the Midwestern US, including Kansas (Rashad 1955), Minnesota (Smart et al. 2016b), North Dakota (Smart et al. 2016b), Indiana (Long and Krupke 2016), Ohio (Sponsler et al. 2017), Wisconsin (Severson 1978). Except for red clover, the other five legume species are also considered to be a significant source of nectar for the production of a honey crop (Sweet 1949).

These six legume species were also recommended (Decourtye et al. 2010) as opportunities for the enhancement of pollen forage in agricultural landscapes. If planted as bee forage, care must be taken because some nonnative legumes, e.g., birdsfoot trefoil (Williams and Smith 2007, Gerla et al. 2012) and sweet clover (Cole 1991, Wolf et al. 2003, Conn et al. 2011) can invade and colonize native habitats (i.e., prairie). Less invasive legumes like white clover, red clover and native legumes like purple prairie clover and partridge pea are more suitable choices for increasing source of pollen for honey bees in agricultural landscapes like Iowa. In addition, legumes used as cover crops can improve soil nutrition by fixing nitrogen *via* root symbiosis, thus contributing to stacked benefits for both agronomical and apicultural management.

In conclusion, we did not observe an effect of low versus high cultivation landscapes, surrounding colonies on the amount and diversity of pollen collected by honey bees. In general, honey bees in central Iowa were able to collect pollen even in landscapes dominated with corn and soybean production (i.e. high cultivation). Regardless of the varying surrounding landscapes, a few species of plants considered as attractive bee forage were consistently discovered by honey bees, primarily multiple species of legumes (mainly clover and partridge pea). This was true even for colonies located in fields in which 90% of the land used within a 1.6 km radius was corn and soybean. Although the diversity of our honey bees' pollen diet was generally low, we

determined that by feeding on a pollen diet consisting only of a two taxa of legumes, honey bees experienced reduced mortality from viral infections. This suggests that even small improvements in forage diversity has the potential for improving the health of honey bees.

If honey bees deliberately acquire pollen from different species to satisfy their nutritional needs (Hendriksma and Shafir 2016), this may have been challenging in the agricultural landscape of central Iowa especially early in the growing season when clover dominates the pollen brought back to colonies (Fig. 5, Supp. Figs. 1-2). Some native plants can be planted in agricultural land dominated by corn and soybean to increase forage diversity in early season. For example, sunflower (*Helianthus annuus*) can have medication effect on bees, and could be an option for forage enhancement (Jonathan et al. 2018, LoCascio et al. 2019). Efforts to conserve beneficial insects have revealed that native plants commonly found in prairies, the dominant habitat in Iowa before European settlement, are highly attractive to native pollinators as well as managed honey bees (Tuell et al. 2008, Blaauw and Isaacs 2014). Many of the flowering forbs found in those prairies, such as native sunflower and goldenrod, may be a potential forage source for honey bees that surfer from lack of pollen availability in later season observed in this study. Dolezal et al. (2019a) demonstrated that prairies can enhance bee colony weight, mainly composed by honey, in later season when those native prairie forbs are blooming, suggesting an improvement in forage availability. To what extent honey bees would benefit from a more diverse community of flowering plants late in the season is not known. Furthermore, it remains to be tested whether honey bees in more diverse landscapes with more accessible floral resources are more efficient at foraging, requiring less energy spent in searching for pollen and nectar.

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Tables and Figures

Table 1. High versus low cultivation Landscapes assessed by proportion of area of crop (i.e., corn and soybean) and non-crop area in the landscape surrounding apiaries within 1.6 Km radius. In each year, five fields were selected to represent each landscape category.

Landscape category	Mean ± SE % of non-crop habitat (range of non-crop habitat)	
	2015	2016
High cultivation	18.40 ± 3.78 (7-26)	13.60 ± 2.64 (7-21)
Low cultivation	54.20 ± 4.72 (44-70)	69.20 ± 8.67 (48-88)

Table 2. Repeated measure ANOVA for pollen abundance and diversity in 2015 and 2016 using linear mixed effects model.

Pollen	Source of variance	D.F.	F value	*P* value
Abundance	***2015***			
	Landscape	1, 10.3	0.13	0.728
	Date	3, 21.6	19.26	< 0.000
	Landscape × Date*	3, 21.6	2.36	0.099
	2016			
	Landscape	1, 15.1	0.64	0.437
	Date	6, 42.2	0.79	0.584
	Landscape × Date	6, 42.2	0.92	0.488
Taxonomic richness	***2015***			
	Landscape	1, 17.4	0.20	0.659
	Date	9, 65.8	6.28	<0.000
	Landscape × Date	9, 65.8	0.38	0.939
	2016			
	Landscape	1, 18.3	0.41	0.528
	Date	12, 82.6	1.33	0.220
	Landscape × Date	12, 82.6	0.37	0.971
Shannon diversity	***2015***			
	Landscape	1, 22.2	0.01	0.929
	Date	9, 65.4	2.71	0.010
	Landscape × Date	9, 65.4	0.36	0.948
	2016			
	Landscape	1, 22.7	1.31	0.265
	Date	12, 86.7	1.95	0.039
	Landscape × Date	12, 86.7	0.64	0.800

* Interaction between landscape and date.

Table 3. Taxa of plants identified in the pollen collected by honey bees during 2015.

Plant taxa*	% of each pollen type (Mean ± SE) by weight	
	High cultivation	Low cultivation
Triflolium repens	42.13 ± 10.66	34.38 ± 15.84
Trifolium pretense	24.66 ± 10.08	32.39 ± 8.64
Chamaecrista fasciculata‡	24.37 ± 8.44	26.77 ± 12.11
Solidago spp.‡	3.10 ± 1.69	2.10 ± 0.85
Circium vulgare	2.15 ± 1.43	0.87 ± 0.38
Lotus corniculatus	0.90 ± 0.86	0.13 ± 0.13
Helianthus, Heliopsis, Silphium spp., ‡	0.63 ± 0.41	0.27 ± 0.19
Sambucus canadensis	0.59 ± 0.59	0
Ambrosia spp.	0.30 ± 0.10	0.64 ± 0.39
Melilotus spp.	0.14 ± 0.14	0.81 ± 0.81
Dalea purpurea‡	0.13 ± 0.11	0.05 ± 0.05
Zea mays	0.06 ± 0.06	0.18 ± 0.18
Saponaria officinalis	0	0.29 ± 0.29
Ratibida pinnata†, ‡	0	0
Phlox paniculat ‡	0	0.24 ± 0.24
Unknown taxa*	0.82 ± 0.43	0.87 ± 0.44

* A total of 18 unrecognized pollen types were combined into “unknown taxa”, but % of each unrecognized pollen type was informed in Supp. Table 1. Pollen types were arranged in the order of high to low percentage of simple landscape.

†Pollen was less than < 0.01%.

‡ Native plants.

Table 4. Taxa of plants identified in the pollen collected by honey bees during 2016.

Pollen type*	% of each pollen type (Mean ± SE) by weight	
	Simple	Complex
Trifolium pretense	34.38 ± 7.33	39.42 ± 9.70
Triflolium repens	30.72 ± 4.45	27.51 ± 7.75
Chamaecrista fasciculata‡,	10.83 ± 6.60	5.71 ± 5.24
Lotus corniculatus	4.69 ± 4.23	1.59 ± 0.96
Melilotus spp.	3.88 ± 1.04	1.94 ± 0.57
Ambrosia spp.	2.09 ± 0.54	3.71 ± 2.07
Circium vulgare	1.76 ± 1.63	0.83 ± 0.44
Dalea purpurea‡	1.42 ± 1.34	0.01 ± 0.01
Zea mays	1.21 ± 0.71	1.93 ± 1.40
Iris versicolor‡	0.19 ± 0.09	1.90 ± 1.87
Taraxacum officinale	0.17 ± 0.13	0.23 ± 0.15
Pastinaca sativa	0.17 ± 0.11	0.16 ± 0.08
Saponaria officinalis	0.15 ± 0.11	3.95 ± 3.63
Tilia americana‡	0.13 ± 0.09	3.63 ± 3.63
Asparagus officinalis	0.12 ± 0.12	0
Solidago spp. ‡	0.08 ± 0.04	0.49 ± 0.45
Daucus carota	0.05 ± 0.03	0.42 ± 0.32
Phlox paniculata‡	0.04 ± 0.04	0.17 ± 0.15
Helianthus, Heliopsis &Silphium spp. ‡	0.04 ± 0.03	0.15 ± 0.11
Cichorium intybus	0.03 ± 0.03	0
Sambucus canadensis	0.03 ± 0.02	0.02 ± 0.02
Rudbeckia hirta	0.02 ± 0.01	0.02 ± 0.02
Zizia aurea‡	0.01 ± 0.01	0
Hemerocallis fulva	0.01 ± 0.01	0.03 ± 0.03
Ratibida pinnata†, ‡	0	0
Verbena stricta‡	0	0.16 ± 0.14
Unknown taxa*	7.78 ± 3.95	8.27 ± 1.58

* A total of 38 unrecognized taxa combined into “unknown taxa”, but % of each unrecognized taxa

was informed in Supp. Table 2.

† Pollen was less than < 0.01%.

‡ Native plants.

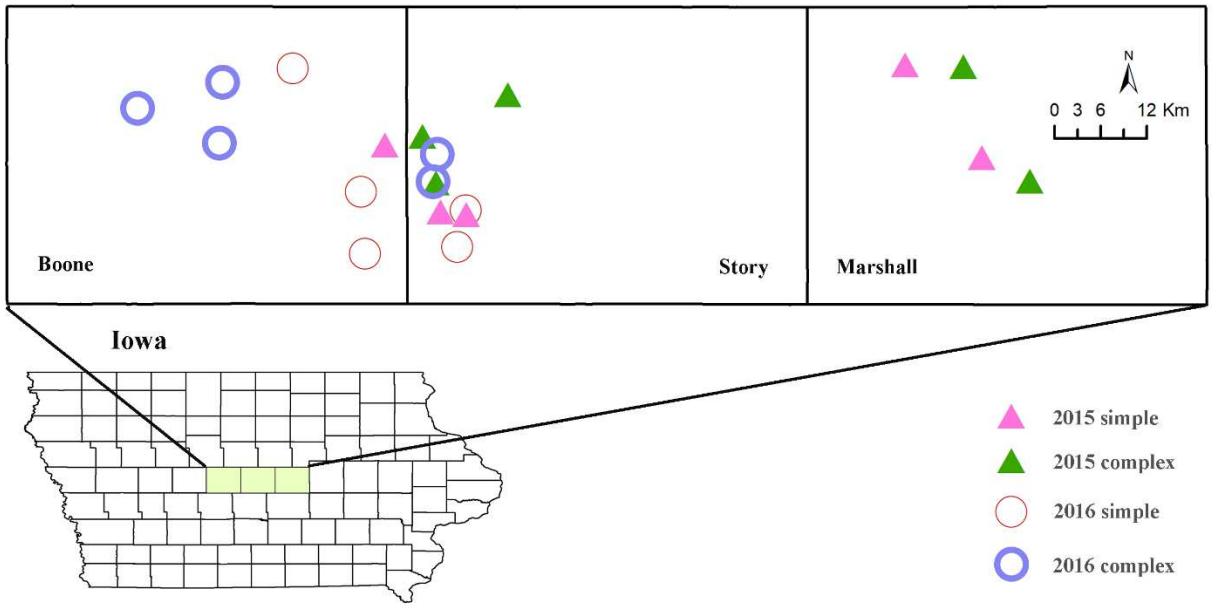


Figure 1. Location of apiaries within three counties of central Iowa, during 2015 and 2016. Apiaries were placed adjacent to soybean fields that were surrounded by landscapes that fitted two landscape categories (high vs low cultivation).

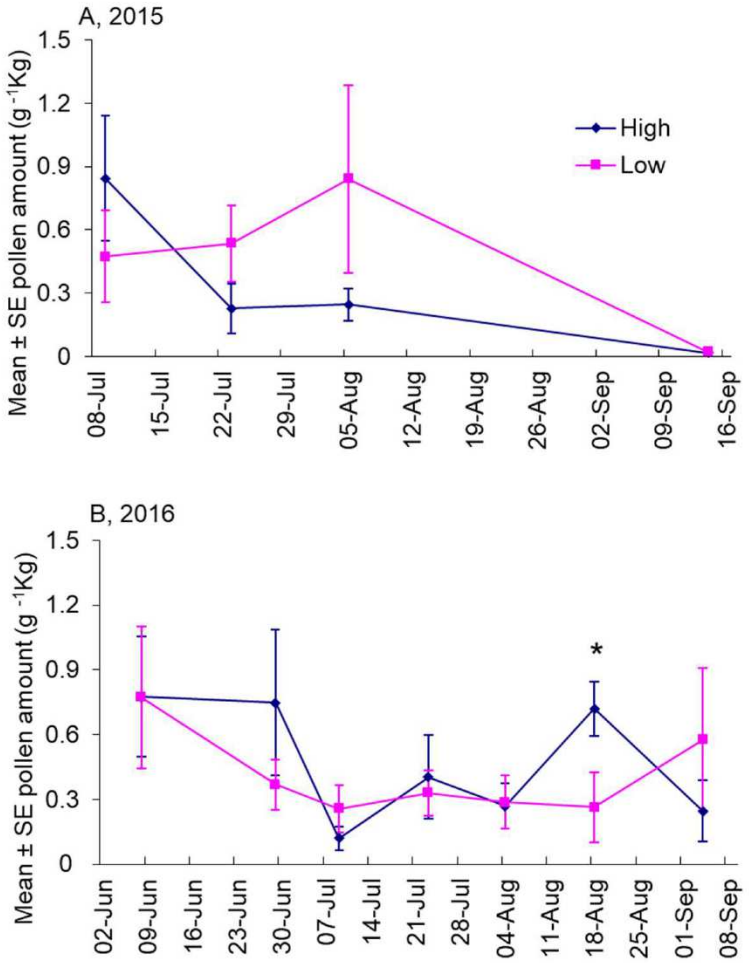


Figure 2. The abundance of pollen collected by honey bees in high and low cultivation landscapes of central Iowa during 2015 (A) and 2016 (B). The weight (g) of pollen collected was normalized by net colony weight (Kg), resulting in the use of $g^{-1}Kg$ along the y-axis. Pollen abundance did not statistically differ between the two landscape categories (Table 2). Note this analysis was conducted on a subset of dates that included only days when colony weight was measured (excluding the pollen data without available corresponding colony weight for normalization).* Indicates a statistically significant difference on a single date by least square means under the mixed effects model ($P < 0.05$).

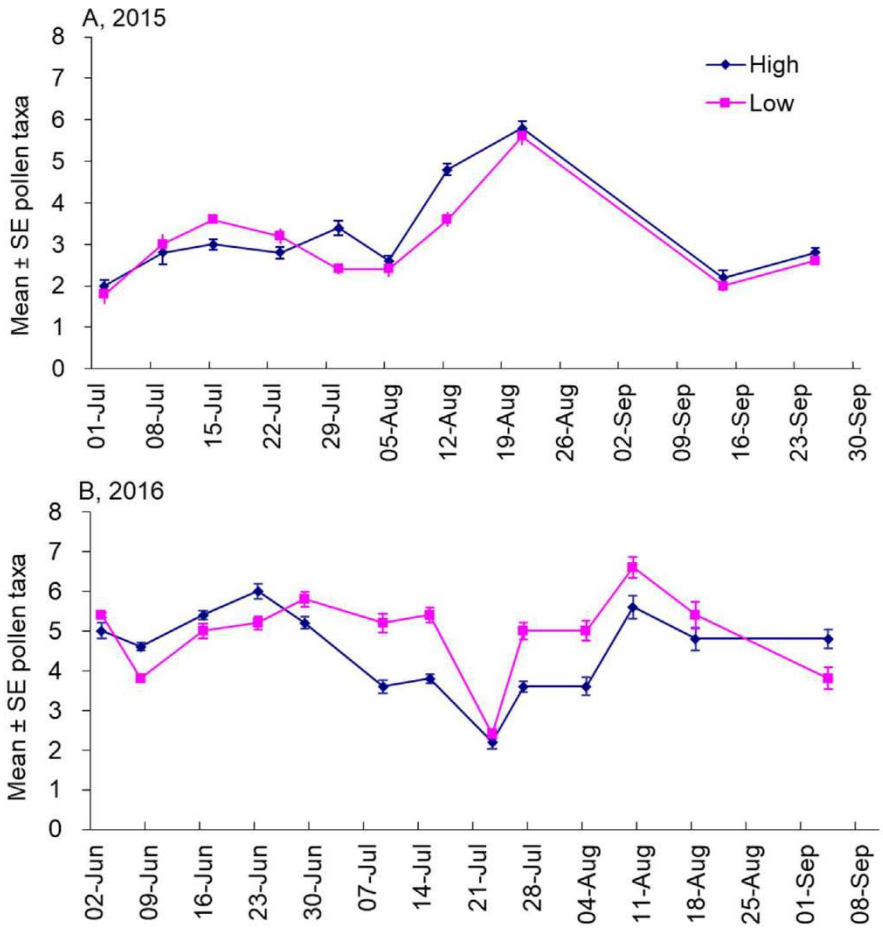


Figure 3. Taxa richness of pollen returned to honey bee colonies in two different landscapes of central Iowa during 2015 (A) and 2016 (B). No significant difference was observed between the two landscape categories (Table 2).

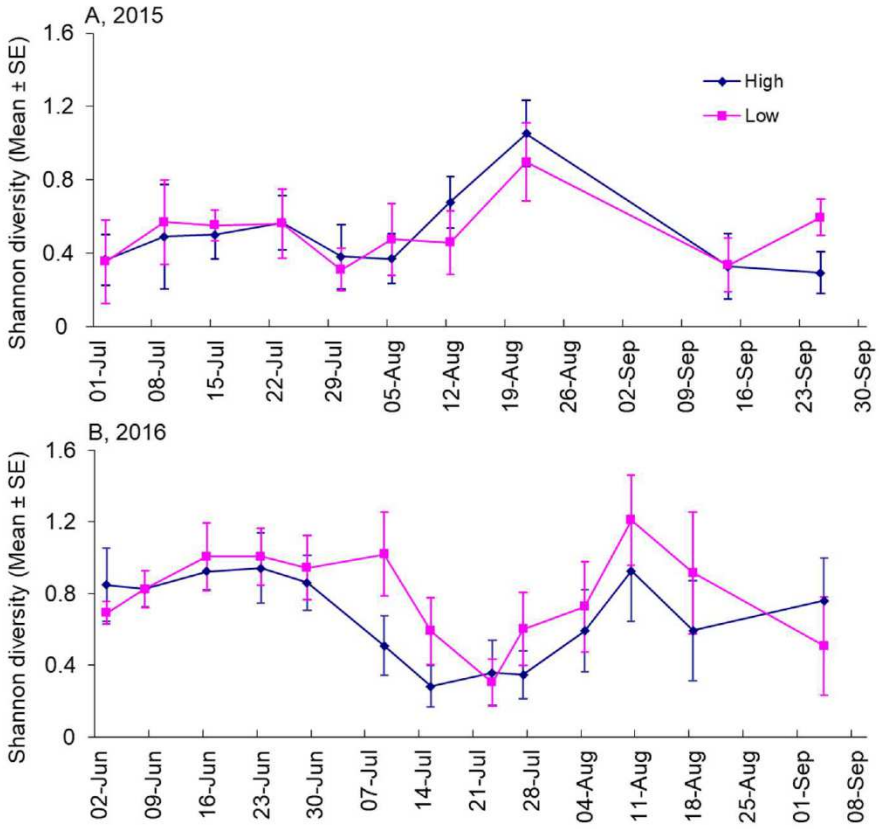


Figure 4. The diversity of pollen returned to honey bee colonies in two different landscapes of central Iowa as estimated with the Shannon diversity index during 2015 (A) and 2016 (B). No significant difference was found between the two landscape categories (Table 2).

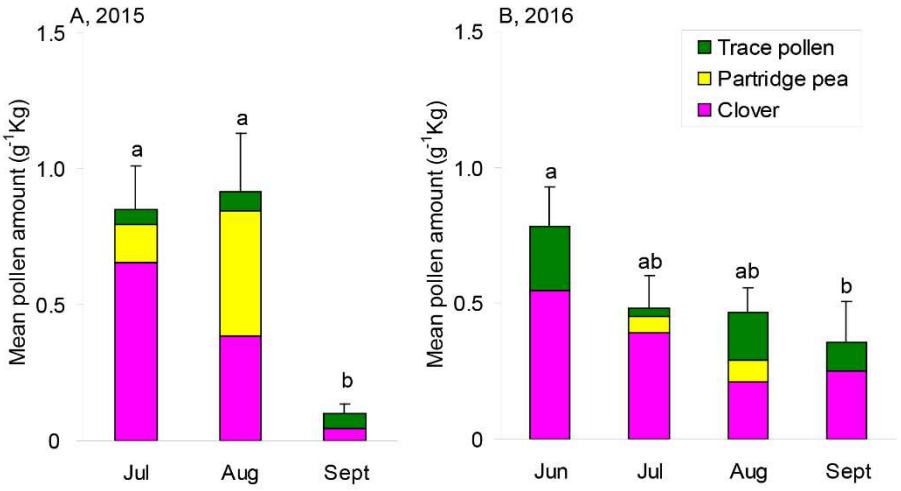


Figure 5. The community composition of pollen collected by honey bees in two categories of central Iowa landscapes by month during 2015 (A) and 2016 (B). Columns represent the total amount of pollen collected and colors within a column indicated plant species. Plants were included in the group of 'trace pollen' if they contributed less than 5% of the total by weight. See Tables 3 and 4 and Supp. Tables 1 and 2 for a list of species represented in the pollen collection. Error bars represent standard errors of averaged total pollen. Different letters above error bars indicated the significant differences of averaged total pollen among months according to ANOVA results followed by Tukey-Kramer HSD multiple comparisons.

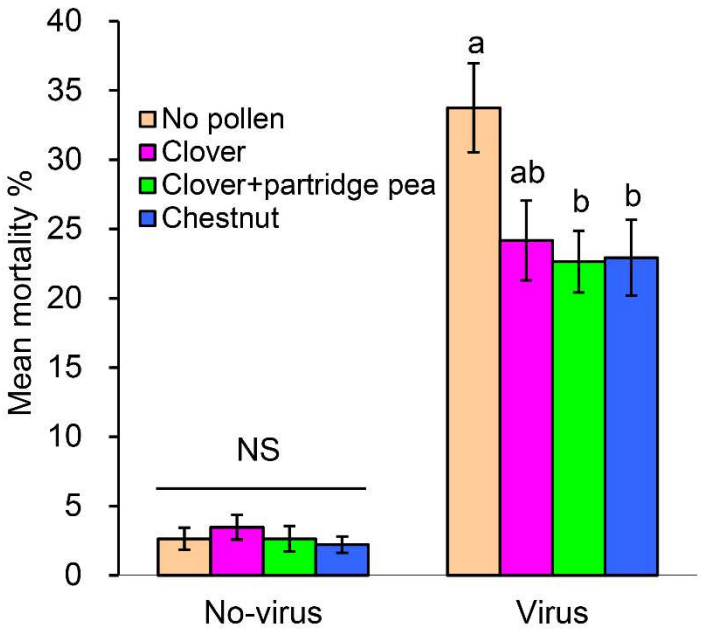


Figure 6. Mortality of caged honey bees either uninfected or infected with a mixture of viruses. Each group of bees were provided four differing pollen diets and ad libitum sucrose solution. Mortality is reported as the percent of individual bees that died after 72 h within each treatment. The mortality of bees infected with virus was significantly higher than uninfected bees (t = 15.39; df = 109.48; $P < 0.0001$). Different letters above the standard error bars indicate significant differences among the diets for virus-infected bees (F = 3.62; df = 3, 95; P = 0.016, multiple comparison by Tukey-Kramer HSD). Mortality of honey bees uninfected with virus did not significantly (NS) differ by pollen diet (F = 3.62; df = 3, 95; P = 0.016).

Supplementary Tables and Figures

Supp. Table 1 Taxa of plants unidentified in the pollen collected by honey bees during 2015.

Unidentified plant taxa (UT) [§]	% of each pollen type (Mean ± SE) by weight		Frequency*
	High cultivation	Low cultivation	
UT1	0.176±0.108	0.0856±0.0398	5
UT2	0.1714±0.168	0	2
UT3	0.1956±0.1839	0.1143±0.1032	4
UT4	0	0.0523±0.0523	1
UT5	0.1404±0.1404	0.0002±0.0002	2
UT6	0	0.0319±0.0319	1
UT7	0.0693±0.0693	0.0732±0.0732	2
UT8	0.0082±0.0082	0	1
UT9	0.0068±0.0068	0	1
UT10	0.0014±0.0014	0.014±0.014	2
UT11	0	0.003±0.003	1
UT12	0.0004±0.0004	0.4961±0.4961	2
UT13	0.0008±0.0008	0	1
UT14	0.0166±0.0166	0	1
UT15	0	0.0031±0.0031	1
UT16	0	0.0003±0.0003	1
UT17	0.012±0.012	0	1
UT18	0.0197±0.0197	0	1

* Frequency was calculated as the number of sites at which a pollen type was found.

[§] UT, unidentified taxa in our pollen collection.

Supp. Table 2 Taxa of plants unidentified in the pollen collected by honey bees during 2016.

Unidentified plant taxa (UT) [§]	% of each pollen type (Mean ± SE) by weight		Frequency*
	High cultivation	Low cultivation	
UT-3	0.501±0.2744	1.013±0.4093	8
UN-2	0.0194±0.0156	0.0423±0.0423	3
UN-13	0.0108±0.0108	0.0334±0.0255	3
UT-19	0.0022±0.0022	0.0343±0.0343	2
UT-20	0.0218±0.0207	0	2
UT-21	0.0376±0.0376	0.0396±0.0396	2
UT-22	0.0617±0.0427	0.0499±0.0499	3
UT-23	0.0215±0.0215	0.0022±0.0022	2
UT-24	0	0.0071±0.0071	1
UT-25	0.0103±0.0103	0.0178±0.0178	2
UT-26	0	0.0593±0.0593	1
UT-27	0.2211±0.1397	0.0085±0.0085	3
UT-28	0	0.0359±0.0359	1
UT-29	0.0902±0.055	0	3
UT-30	0.1605±0.1605	0.0646±0.0588	3
UT-31	0.1026±0.0919	0.6399±0.6311	5
UT-32	0.0488±0.0488	0.0471±0.0471	2
UT-33	0	0.0033±0.0033	1
UT-34	0	0	0
UT-35	0.0101±0.0101	0.398±0.386	3
UT-36	0.0252±0.0252	0	1
UT-37	0.0021±0.0021	0	1
UT-38	0	0.0009±0.0009	1
UT-39	0.0352±0.0352	0.0489±0.0277	4
UT-40	0	0.0263±0.0218	2
UT-41	0	0.0076±0.0076	1
UT-42	0	0.0905±0.0826	2

Supp. Table 2 Continued.

Unidentified plant taxa (UT) [§]	% of each pollen type (Mean ± SE) by weight		Frequency*
	High cultivation	Low cultivation	
UT-43	0.0559±0.0314	1.6572±1.4738	7
UT-44	0.2371±0.1467	0.2267±0.1279	6
UT-45	0	0	0
UT-46	3.8165±3.3079	0.0256±0.0256	3
UT-47	0.0702±0.0702	0	1
UT-48	1.9748±1.3745	0.3886±0.2556	6
UT-49	0.0059±0.0059	0	1
UT-50	0.2279±0.2279	0.1717±0.1717	2
UT-51	0	0	0
UT-52	0	0.0268±0.0268	1
UT-53	0	0.1476±0.1476	1
UT-54	0.0109±0.0109	0.0206±0.0206	2
UT-55	0	0.2423±0.2423	1
UT-56	0	0.4409±0.4409	1

* Frequency was calculated as the number of sites at which a pollen type was found.

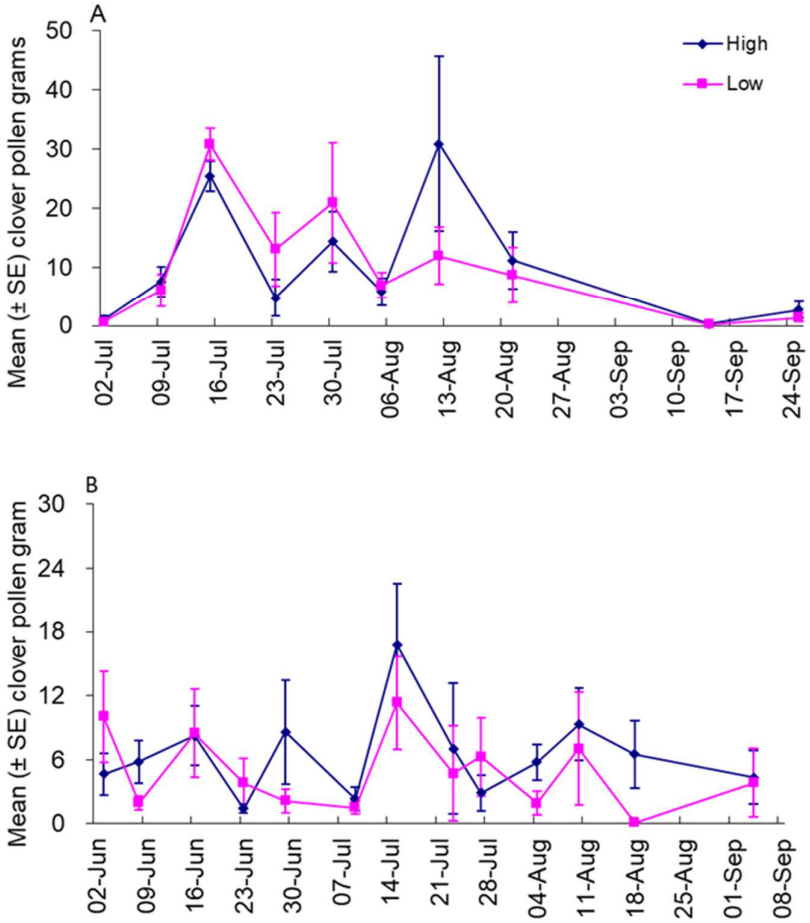
[§] UT, unidentified taxa in our pollen collection.

Supp. Table 3 A summary of number of plant taxa found in pollen collected by honey bees in 2015 and 2016.

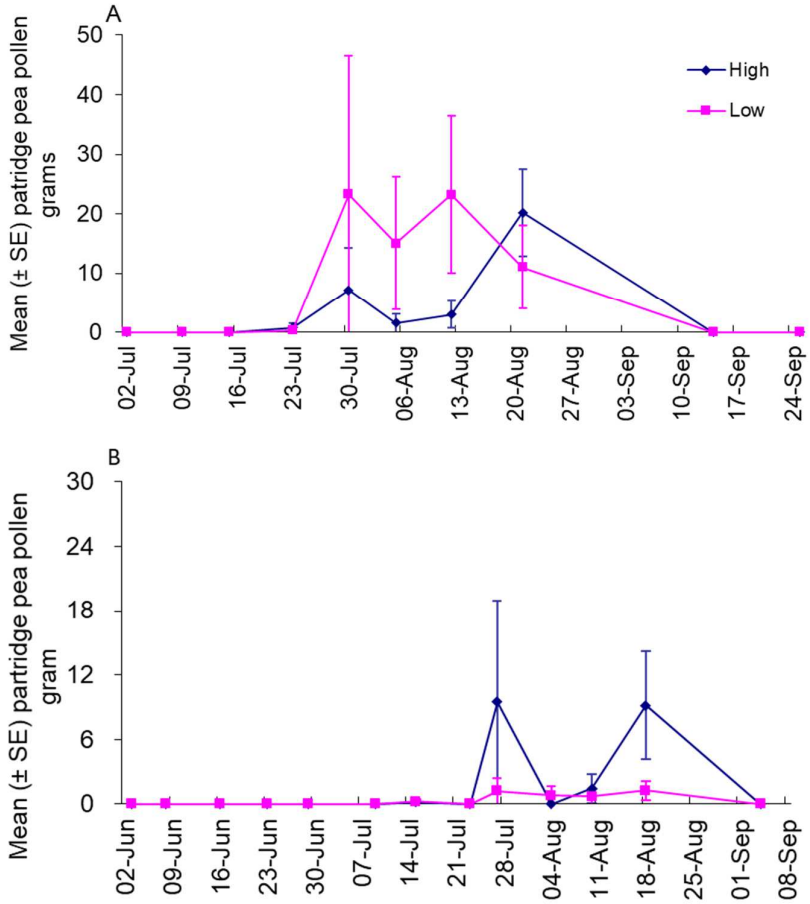
Identification	Year	Total Taxa	Shared taxa*	Unshared taxa	Number of Taxa in two landscapes		Cropland**		Grassland**		Woodland**	
					High	Low	High	Low	High	Low	High	Low
Total taxa	2015	33	17	16	25	25						
Identified taxa	2015	15	11	4	12	14	8	8	4	6	0	0
Unidentified taxa	2015	18	6	12	13	11						
Total taxa	2016	64	41	23	51	54						
Identified taxa	2016	26	21	5	25	22	17	12	7	9	1	1
Unidentified taxa	2016	38	20	18	26	32						

* Taxa shared between low vs high cultivation landscapes.

** Number of taxa associated with potential land use types found in each of the two landscape categories.



Supp. Fig. 1 The amount of clover pollen collected by honey bees in two landscape classes in central Iowa during 2015 (A) and 2016 (B). There was no significant difference in the amount of pollen collected between the two landscape categories in both years (2015: F = 0.16, df = 1, 17.2, $P = 0.695$; F = 1.91, df = 1, 23.9, $P = 0.180$). The amount of pollen reported in the figure is based on the data without normalization by colony weight.



Supp. Fig. 2 The amount of partridge pollen collected by honey bees in two landscape classes in central Iowa during 2015 (A) and 2016 (B). There was no significant difference in the amount of pollen collected between the two landscape classes in both years (2015: F = 0, df = 1, 18.7, $p = 0.948$; F = 0.16, df = 1, 17.2, $p = 0.695$). The amount of pollen reported in the figure is based on the data without normalization by colony weight.

CHAPTER 3. VARIATION IN ANNUAL WEATHER, RATHER THAN LAND USE, AFFECTS HONEY BEE POLLEN COLLECTION IN AN AGRICULTURAL LANDSCAPE

Modified from a manuscript under review in Journal of Apicultural Research

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Abstract

Variation in land use and climate can affect the foraging of honey bees. For example, agricultural intensification has reduced the reliable availability of forage for honey bees. Foraging behavior by honey bees is affected by aspects of the climate, like variation in temperature and rainfall, both factors that affect flight. To what extent variation in land use and climate explains the abundance and diversity of pollen collected by honey bees is not well studied. Through a multiyear study across three land use types, including soybean fields, diverse fruit and vegetable (DFV) farms, and prairies in central Iowa, USA, we explored if land use and extreme weather (i.e., high temperature and meteorological drought) affected the abundance or diversity of pollen collected by honey bees. We placed sentinel colonies in fields within these types during 2015-2017. Samples of pollen from these colonies were collected during June to September, and pollen abundance and plant diversity represented in

pollen was determined using microscopy. The total amount of pollen collected by honey bees did not differ among the three land use types, neither did plant diversity represented in pollen. During years when high temperatures occurred concurrently with a meteorological drought in July, the amount of collected pollen was reduced. Forage may be improved by re-integration of native floral resources, such as golden rod (*Solidago* spp.) and partridge pea (*Chamaecrista fasciculata*), favored by honey bees that are resilient to climate change.

Key words

Growing degree days, rainfall, clover, *Apis mellifera*, climate change

Introduction

Shortage in forage caused by loss of natural and semi-natural habitat in agricultural landscapes contributes to high colony losses of honey bees (*Apis mellifera*) in both the USA and Europe (Carreck et al. 1997, Naug 2009, Neumann and Carreck 2010, Potts et al. 2010, vanEngelsdorp and Meixner 2010, Scheper et al. 2014, Goulson et al. 2015, Paudel et al. 2015, Steinhauer et al. 2018). The Midwestern USA is an example of forage loss due to agricultural intensification, which has been reported to negatively impact honey bee health (Wright and Wimberly 2013, Otto et al. 2018, Dolezal et al. 2019). Pollen is an extremely important component of the honey bee diet, providing the majority of their dietary protein/amino acids, lipids and micro-nutrients (Black 2006, Vaudo et al. 2015, Wright et al. 2018). Honey can also contain those nutrients, but only in trace levels (Bogdanov et al. 2008). Pollen abundance, diversity, and nutritional quality may also vary widely throughout a growing season, between years, and under different land use types. Although there have been

several studies on the impacts of land use on pollen collection by honey bees (Colwell et al. 2017, Danner et al. 2017), most published studies present short-term datasets that may not capture the long-term (within a year and across years) dynamics of pollen collected by honey bees in a given area.

In the context of highly farmed landscapes, uncultivated land adjacent to honey bee colonies may produce a more diverse community of plants, which in turn can be a diverse source of pollen collected by honey bees to alleviate shortages after the surrounding crop land has completed anthesis. Honey bee responses to land use are highly variable, with conflicting results that depend upon the surrounding matrix. For example, abundance and diversity of pollen collected by honey bees did not vary with the proportion of the landscape committed to natural or semi-natural habitats (Danner et al. 2017). In contrast, placing colonies on fallow land allowed honey bees to collect more diverse pollen than those kept on land committed to fruit production (Colwell et al., 2017). To what extent the differences in the adjacent crop and community of plants in the surrounding landscape explains the differences in these studies is not clear.

The state of Iowa in the Midwestern USA is an interesting area to study the effects of landscape on honey bee forage because it represents a zenith of monoculture-based agricultural production. A diverse community of native perennial grasses and forbs that historically co-existed within prairies throughout much of the Midwestern USA has been replaced by production of annual crops (Samson and Knopf 1994). There is increasing interest in agricultural and ecological landscape diversification as a way to support pollinator

health (Aizen et al. 2019). Prairie plants have the potential to enhance pollinator abundance and diversity (Tuell et al. 2008, Blaauw and Isaacs 2014), but their occurrence in the Iowa landscape is limited to roadsides, and a few remnant and reconstructed prairies (Ries et al. 2001, Reeder and Clymer 2015). Another possible strategy to increase landscape diversity to enhance pollinators and pollination is through more diverse agriculture. Although the majority of Iowa farmland is committed to the production of corn and soybean (average farm size of 106 ha), there are approximately two thousand smaller farms (average farm size < 3 ha) producing fruits and vegetables throughout the growing season (USDA-NASS 2019b). Fruit and vegetable farms in Iowa can provide subtle improvements to colony health (St Clair et al. 2020), but the extent to which honey bees use components of these farms for forage is unknown. Like other pollinators, honey bees may benefit from foraging at both diverse fruit and vegetable (DFV) farms as well as prairies.

In addition to natural and semi-natural habitat loss, there is growing concern for the effects of climate change on bee health and the availability of forage (vanEngelsdorp and Meixner 2010, Goulson et al. 2015). The amount of pollen produced by plants (Bonny 1980, Nilsson and Persson 1981, Emberlin et al. 1993) and pollen collected by honey bees (O'Neal and Waller 1984, Requier et al. 2015, Danner et al. 2017) varies by season or year. Extreme fluctuations in weather patterns (Wuebbles et al. 2017) could potentially lead to variation in forage availability that negatively impact honey bees. Increased temperature and reduced rainfall associated with changing climate are anticipated to reduce plant diversity (Moran et al. 2014, Scheper et al. 2014, Harrison et al. 2015), potentially altering forage available to

honey bees. Another aspect of climate change, the occurrence of extreme weather events (e.g. meteorological droughts, below-average precipitation during prolonged period) can reduce pollen or nectar production of plants used by honey bees for forage (vanEngelsdorp and Meixner 2010). In landscapes with diverse floral resources, honey bees may be buffered from climate-induced shifts in forage availability due to their polyphagous feeding habits. For example, honey bees may be able to shift from using host plants affected by certain forms of climate-change induced stress (e.g. drought-sensitive) to others (e.g. drought-resistant). However, if limited floral resources in an agricultural landscape are further constrained by severe weather, honey bees may be more susceptible to suffer the negative impacts of forage dearth. Efforts to restore or reconstruct habitat for pollinator conservation without considering the consistency of the plant community over time may limit the value of these efforts for conserving honey bees and other pollinators. Studying how forage used by bees varies across multiple years with fluctuating annual weather can help identify how honey bees respond to a changing climate.

To better understand how honey bee forage varies by land use, across seasons and years, we constructed a multi-year experiment using sentinel apiaries deployed at key land use types in the highly-farmed agroecosystem of central Iowa, in the Midwestern USA. Our goal was to determine if and how variation in land use affected the diversity and abundance of pollen collected by honey bees. In 2016, we selected farms of two types (monoculture soybean or DFV) as well as prairies in central Iowa, to compare the effects of land use on honey bee pollen collection. To determine if there was significant year-to-year variation in

the pollen returned to colonies, we kept bees at soybean fields during a three-year period (2015-2017). This multi-year, multi-site monitoring of honey bee forage in the context of a highly farmed agroecosystem allowed us to better document the environmental drivers of variation in pollen collected by honey bees.

Materials and Methods

Sites and honey bee colonies

A total of 32 research sites were distributed across four counties (Boone, Marshall, Polk, and Story) in Iowa, USA (Fig. 1). This specific region of central Iowa is dominated by agricultural land, with approximately 64% of area comprised by these counties used for soybean and corn production (USDA-NASS 2019a). We identified sites for two purposes: first, to compare diversity and abundance of bee-collected pollen between land uses (soybean fields, diverse fruit and vegetable [DFV] farms and prairies) in only 2016 when sites of these three land uses were chosen; and second, to compare abundance and diversity of bee-collected pollen across years (2015-2017) at soybean fields (Table 1).

Honey bee colonies were housed in conventional Langstroth hives located at either a field margin of a soybean field, DFV farm or a prairie. All the colonies consisted of Italian honey bees (*Apis mellifera ligustica*), and the number of colonies per site varied from one to four and the dates on which data were collected varied by year (Table 1). No supplemental food (sugar solution or pollen patty) was provided to colonies in any year. Varroa mite infestations were treated with thymol (Apilife Var, Chemicals Laif SPA, Vigonza, Italy) in end of August or the beginning of September to prevent late season infestations.

Pollen collection and identification

Each site had one colony with an entrance pollen trap (Brushy Mountain Bee Supply, Wilsonville, USA) in 2015-2016 and two colonies with pollen traps in 2017 (Table 1). Pollen was collected during a 24 h period, selected based on weather forecasts considered favorable for honey bee foraging (e.g. no precipitation). The weight of pollen collected within a trap was recorded. If two traps were present, the weight of each was measured separately and mean weight was used for statistical analysis. To account for the variation in pollen abundance due to varying colony size, the average monthly pollen weight was normalized by the average monthly colony weight (Supp. Fig. 1). Each colony was weighed once to twice a month during regular colony inspections following methods from Dolezal et al. (2019) (Table 1). The colony weight was calculated by taking the difference between the whole colony weight (including wooden components, bees, food stores and wax) and the wooden components (including wooden hive box, lid, bottom board and frames). After collection and weighing, pollen was stored at -20 °C for taxonomic identification.

The diversity of plants represented in the pollen was reported and used to calculate Shannon diversity index to account for both taxon richness and evenness. To determine pollen diversity, 2 g pollen was extracted from each pollen sample collected per site per date and each pollen pellet sorted by color. If a site had two pollen traps, pollen from the two traps was mixed and a 2 g subsample extracted from the mix for identification. This sorted pollen was weighed, dissolved in Calberla's fluid, and then mounted onto glass slides. Plant taxon identification was carried out by examining pollen grains under a light microscope with 200-

magnification, comparing each sample with a reference collection of pollen images from plants collected in our research sites. Bee-collected pollen was identified to the lowest possible taxonomic unit. Pollen that could not be assigned to a taxon were given a morphospecies identification such that each had an individual designation. Taxon richness of plants represented by the pollen was reported for each sampling date. We calculated a Shannon diversity index to estimate plant diversity represented within bee-collected pollen for each site.

Temperature and rainfall

Variation in temperature and rainfall has the potential to affect honey bee foraging activity, and can also directly affect plant growth and development, including pollen production that can be collected by honey bee for diets. To address this important source of variation in pollen collection, first, we estimated if high summer temperature exceeded the optimal growth temperature of plants and if low rainfall triggered a meteorological drought that can potentially suppress the pollen production that is collected by honey bees. Second, we performed a linear regression of temperature or rainfall with pollen collection to identify the trend of this negative impact due to high temperature and low rainfall.

Temperature fluctuates within a day and we used monthly growing degree days (GDD) as an indicator of the effects of temperature on plant growth and development (Kadioğlu and Şaylan 2001, Thuiller et al. 2005). This measurement takes two key temperature points (including maximum and minimum temperature) into consideration as well as a base temperature that is the minimum temperature for a plant species to initiate

growth. Maximum and minimum temperature of an area can be monitored by local weather stations and base temperature for a plant species be measure by experiments. The formula for calculating daily GDD was 1/2 (maximum temperature + minimum temperature) – base temperature. Monthly GDD was a sum of daily GDD within a month.

Each plant species has a range of optimum temperatures for growth and development; if temperatures surpass the upper limit of this optimum temperature, plants are negatively affected. By comparing monthly GDD with an estimate of the upper limit of optimum monthly GDD for flowering plants (see description below), we explored whether high temperatures explain variation in bee-collected pollen amounts in 2015-2017. The upper limit of optimum GDD was calculated with the following equation: 1/2 (upper limit of optimum temperature + minimum temperature) − base temperature. The upper limit of monthly optimum GDD was calculated as a sum of the upper limit of daily optimum GDD across all days within each month.

Although multiple weather stations are run in each county of central Iowa, temperature data were collected from three weather stations (USC00130200 in Boone County, USW00094988 in Marshall County, and Story USW00094989 Story County) that are nearest our research sites thus are most likely to collect data on weather that would affect foraging of our honey bees. These data were downloaded from the website of the National Centers for Environmental Information (National Oceanic and Atmospheric Administration, Asheville, USA).

We lacked information for the upper limit of optimum temperature and base temperature for most wild flowers. To simplify our analysis, we used 30°C as the upper limit of optimum temperature and 10°C as the base temperature for the plant community in our study; both are representative for many grasses, row crops, vegetables, and fruits (Ostrowski 1972, Backlund et al. 2008, Hatfield and Prueger 2015).

We also determined whether meteorology drought occurred in our study area, to test for the potential negative effects of low rainfall on pollen production of flowering plants as pollen diet source for honey bees. Duration of drought can vary from weeks to decades, and in our study, meteorology drought was evaluated by month. We define occurrence of meteorology drought as monthly rainfall that was lower than the average historical monthly rainfall. Rainfall data were extracted from the three weather stations described above. Monthly rainfall was calculated as the cumulative daily rainfall within a given month. Because rainfall were only monitored for the past 20 years across all of those stations, average historical monthly rainfall was calculated within this 20-year period (1998-2017).

Statistical analysis

We used analysis of variance (ANOVA) to compare abundance, taxon richness and the Shannon diversity index of bee-collected pollen between land use types within 2016 only with Proc GLM of SAS 9.3 (Cary, North Caroline, USA). A monthly average was calculated for pollen abundance, richness and Shannon diversity index and used for all analyses (Table 1).

We used a linear mixed model AONVA to explore the linear regression of pollen abundance with GDD or rainfall through Proc Mixed of SAS 9.3 using the pollen collected at soybean field during 2015-2017. The model consisted of pollen abundance (the monthly average pollen weight normalized by the monthly colony weight) as the response variable, monthly GDD and rainfall as the explanatory variable (treated as continuous variables), with year and county as random effects. The coefficients of the parameter (Monthly GDD and rainfall) was estimated and AIC value reported using this model. Because an interaction of each month with GDD and rainfall can not be estimated by one model, this linear mixed model was run on each month in our study. The data were base-10 log transformed to increase normality when necessary.

Results

No variation in pollen collected by land use type

Pollen collection from soybean field, DFV farm, and prairie was compared only in 2016, as it was the only year where we kept colonies at all land use types. Overall, colonies kept at the three land use types in 2016 collected pollen from 84 taxa of plants. Pollen from clover (*T. pretense and T. repens*) and partridge pea (*C. fasciculata*) were the most common types collected by honey bees during the growing season (Table 2, Fig. 2A-D). More than 90 % of pollen mass at each land use type was comprised of 10 plants (Table 2). To demonstrate the composition of pollen in each month, pollen were grouped into three general categories. The first two general categories included two common sources (clover and partridge pea) that were > 5 % by weight. The third general category was trace pollen source

that were composed by other species that was < 5 %. Clover was a common pollen source from June through September; partridge pea was a common pollen source from July to August. In addition to collecting common pollen types, honey bees also continuously used trace pollen across months.

The total amount of pollen collected by honey bees did not differ by land use type during any month (June: $F = 0.80$, df = 2, 17, $P = 0.170$; July: $F = 3.38$, df = 2, 17, $P = 0.059$; August: $F = 0.87$, df = 2, 17, $P = 0.438$; September: $F = 0.38$, df = 2, 15, $P = 0.779$; multiple comparison by Tukey HSD) (Fig. 2 A-D). The number of plant species represented within these pollen samples also did not differ by land use type during any month (June: $F = 2.43$, df = 2, 17, $P = 0.118$; July: $F = 1.49$, df = 2, 17, $P = 0.253$; August: $F = 0.35$, df = 2, 17, $P = 0.712$; September: $F = 1.10$, df = 2, 15, $P = 0.354$) (Fig. 2 E-H). Shannon diversity of pollen, accounting for both the richness and evenness, also did not significantly differ among land uses during any month (June: $F = 2.05$, df = 2, 17, $P = 0.159$; July: $F = 0.18$, df = 2, 17, $P = 0.834$; August: $F = 2.24$, df = 2, 17, $P = 0.137$; September: $F = 0.81$, df = 2, 15, $P = 0.463$) (Fig. 2 I-L).

Variation in pollen collected by year

To study the annual variation in pollen collected by forager bees, we focused on data sets from soybean fields (not the other two land use types) from three consecutive years. Clover and partridge pea were the most common plants represented in the pollen across multiple years (Table 3, Fig. 3A-D). To demonstrate general pattern of pollen composition in each month, the pollen types that were > 5 % by weight were grouped into separate general

categories, including four common pollen sources (clover, partridge pea, golden rod [*Solidago* spp.], sunflowers [*Helianthus & Siphilium* spp.]). The pollen types that was < 5 % were included in only category, i.e. trace pollen. Across the three years, clover was a common pollen source from June through September, except for August 2017, and partridge pea was a common pollen source from July to August. Sunflower was a common pollen source for honey bees only in July 2017. Golden rod was also the common pollen source in July and September 2017. Honey bees continuously used trace pollen across months.

The amount of pollen did not significantly differ among years for any month (June: $F = 2.19$, df = 1, 10, $P = 0.170$; August: $F = 1.76$, df = 2, 19, $P = 0.199$; September: $F = 0.89$, df = 2, 18, $P = 0.427$), except July (Fig. 3A-D). In July, the amount of pollen collected in 2015 was significantly higher than that collected in 2016 and 2017; this amount was not significantly different between 2016 and 2017 ($F = 1.76$, df = 2, 19, $P = 0.018$; multiple comparison by Tukey's HSD).

The taxa richness of pollen collected did not significantly differ among years for any month (June: $F = 0.31$, df = 2, 10, $P = 0.593$; August: $F = 0.83$, df = 2, 19, $P = 0.4527$; September: $F = 1.1$, df = 2, 18, $P = 0.353$), except July (Fig. 3E-H). In July, the taxa richness of pollen collected in 2017 was significantly higher than that of 2015 (July: $F = 5.12$, df = 2, 19, $P = 0.017$; multiple comparison by Tukey's HSD); while the taxon richness in 2016 was intermediate between 2015 and 2017.

The Shannon diversity index did not statistically differ among years for any month (June: F = 0.31, df = 2, 10, $P = 0.587$; August: F = 2.22, df = 2, 19, $P = 0.136$; September: F

= 1.18, df = 2, 18, P = 0.352), except for July (Fig. 3I-L). Also in July, the Shannon diversity index of bee-collected pollen was greater in 2017 than that in 2015 and 2016 (F = 4.2, df = 2, 19, P = 0.031; multiple comparison by Tukey's HSD).

Relationship of pollen with temperature and rainfall

We first determined whether the monthly GDD exceed the upper limit of optimum GDD to confirm whether the potential negative of high temperature would occur. In June of 2016, the monthly GDD (408 °C) was also higher than the upper limit of the monthly optimum GDD (405 °C) (Supp. Table 1). The monthly GDD of July was highest in 2017 (Fig. 4), i.e. 437 °C, which was higher than the upper limit of the monthly optimum GDD (430 °C) (Supp. Table 1). However, the absolute value of monthly GDD (437 °C) in July 2017 was much higher than that (408 °C) in June 2016. In no other month did the monthly GDD exceed the upper limit of the optimum monthly GDD. Furthermore, for the month of July, a linear regression analysis revealed a negative relationship between pollen abundance and increasing GDD (P = 0.0232, Table 4, see Fig 3b)

We also determined whether meteorology drought occurred in our research sites to estimate the potential negative effect of low rainfall on pollen production of flowering plants. We observed a remarkable amount of variation in rainfall around our study sites during the three-year period, i.e. 2015-2017 (Fig. 4, Supp. Table 2). The monthly rainfall for June 2016 (34.02 mm) and 2017 (72.5 mm) was 73.25 % and 57.01 % lower than the 20-year average (127.17 mm), respectively (Fig. 4, Supp. Table 2). The lowest monthly rainfall for the month of July occurred in 2017 (71.6 mm) which was 26 % lower than the 20-year average (96.7

mm). The lowest monthly rainfall for the month of August also occurred in 2017 (103.3 mm) and was 13.59 % lower than the 20-year average (119.54 mm). We considered those months in which the rainfall was below the 20-year average to have experienced meteorological drought. In summary, meteorological drought did not occur in 2015 and a one-month (June) drought occurred in 2016. A meteorological drought occurred during a three month period (June through August) in 2017. Furthermore, we found a negative relationship of pollen abundance with decreasing rainfall (i.e. positive relationship with increasing rainfall) (P = 0.0114, Table 4; see Fig. 3F) through a linear regression analysis using a linear mixed model ANOVA.

Discussion

Pollen collected by bees did not vary with land use

Overall, we found no effect of land use type (soybean field, DFV farm or prairie) on abundance and diversity of pollen collected by honey bees in the highly-farmed agroecosystem of Iowa. This finding is surprising, given the fact that visual inspection of these sites demonstrated that the floral resources around colonies were locally improved at DFV farms and prairies compared to soybean fields (Supp. Fig. 2 & Supp. Table 3). Regardless of land use, honey bees consistently collected most of their pollen from a small number of common plant species (i.e. *Trifolium pretense*, *T. repens*, *C. fasciculate*) found within the larger surrounding landscapes. This suggests that the surrounding landscape matrix, rather than the smaller area of specific land use in the direct vicinity of the apiary, is the primary source of pollen forage. There were many more plants flowering than these

legumes, especially at DFV farms and prairies. That legumes were a major source of pollen may reflect a preference by honey bees for foraging on those plants.

Our results demonstrate that the floral resources in DFV farms and prairies, while appearing more abundant based on visual inspection (Supp. Fig. 2), did not result in more pollen returned to the colonies. These differences in floral resources may have resulted in more nectar collection. Colonies used for this study were monitored in another experiment to estimate the impacts of soybean fields versus DFV farms on colony growth (St Clair et al. 2020). Colonies were heavier at DFV farms than soybean fields during the growing season, indicating more nectar was collected at DFV farms. Blooming soybeans can be found throughout the landscapes in which these colonies were kept, but honey bees kept at DFV farms may have taken advantage of nectar from the diverse crops and weedy plants at DFV farms. Those results suggest honey bee response to land use types differ in their collection of nectar versus pollen.

Bee-collected pollen differed among years and was related to extreme weather

Our results suggest that the abundance of bee-collected pollen was negatively affected by increasing temperature and decreasing rainfall. This relationship was most noticeable in July of 2017, when high temperatures (exceeding the upper limit of optimum monthly GDD) and meteorological drought co-occurred. Neither high temperature nor meteorological drought occurred in July of 2015 and 2016. Temperature and rainfall in July 2016 was intermediate between 2015 and 2016, indicating a moderate effect on pollen abundance.

In the short term, high summer temperatures can negatively affect plant productivity (Backlund et al. 2008, Izaurralde et al. 2011, Hatfield and Prueger 2015), which may account for decreased pollen amount collected by honey bees, because the occurrence of flowering and pollen shed are transitional periods of vegetative growth to reproductive growth that can be affected by overwhelming high temperature (Cleland et al. 2007). High temperatures at a more severe level can cause death of drought- and heat- susceptible plants, leading to dramatic decline in the floral resource used by bees. For example, high temperatures are known to negatively affect red clover (*T. pretense*) and white clover (*T. repens*) populations (Clark and Harris 1996, Woodfield et al. 1996), two of the most common plants used by honey bees for pollen in our study.

Low rainfall can worsen the negative impact of high temperature on growth and development of white clover (Woodfield et al. 1996). Without sufficient water, the plants are more susceptible to high temperature, thus, the upper limit of the optimum temperature for white clover can decrease from 30 °C to as low as 25 °C (Mitchell and Lucanus 1962, Ostrowski 1972, Fukai and Silsbury 1977, Clark and Harris 1996, Woodfield et al. 1996, Black et al. 2009). In our study, we estimated the upper limit of the optimum monthly GDD for white clover was 430 °C, which was exceeded by the actual monthly GDD (437 °C) in July of 2017. This estimate was based on normal conditions without considering the potential effects of meteorological drought. However, when meteorological drought co-occurred with high temperatures, the upper limit of optimum monthly GDD for white clover could be as low as 352 °C, which was much lower than what we estimated. The impact of both

temperature and rainfall was most noticeable in reduction in clover pollen collected during July 2017, which among the most abundantly collected pollens in the rest our study (Supp. Table 4).

Although we lack data for the effect of the meteorological drought on changes in the optimum temperature for other flowering plants, the low amount of pollen collected from those plants during July 2017 suggests they may also be negatively affected. Significantly less clover and other pollen types resulted in the overall decrease in pollen collected in July. The higher diversity of pollen collected during July 2017 suggests that honey bees may have changed their foraging preferences to meet their pollen needs. We found honey bees used Golden rod (*Solidago* spp.) and sunflowers (*Helianthus & Siphilium* spp.) were fit into the trace category in 2015 and 2016, but were major pollen sources in 2017, suggesting a change of foraging activity. These plants were a common source of pollen throughout the hot and dry July of 2017, suggesting they are sufficiently drought- and heat-tolerant to rescue honey bees from a lack of forage.

The negative effects of extreme weather on flowering plants and bee foraging may extend beyond the weather event itself. In our study, bee-collected white and red clover pollen was limited during July 2017 and totally disappeared in August 2017 (Fig. 3C-D, Supp. Table 4). The August 2017 decline cannot be attributed to clover phenology, since red and white clover were abundant in both August 2015 and 2016. Cooler temperatures in August 2017 did not alleviate the shortage of clover pollen, suggesting populations of clover were suppressed beyond the actual hot and drought period.

When high temperature and meteorological drought happen simultaneously, plants resistant to unfavorable weather may serve as a "nutritional reservoir" for honey bees, allowing them to reach the minimum amount of pollen required to meet colony nutritional needs. Partridge pea (*C. fasciculata*) is considered a drought-resistant plant (Houck and Row 2019) and provided a substantial pollen resource to honey bees in the post-drought period of August of 2017, possibly preventing a disastrous dearth in pollen. The high percentage of partridge pea pollen in both wet years (2015) and dry years (2017) indicates that this plant may be adapted to both dry and wet climates, suggesting it may be a climate-resilient choice for supplementing the landscape to improve honey bee forage.

In the long term, a warming and drying climate can reduce regional or global plant diversity (Harrison et al. 2015). Increasing global temperatures in the USA and many other parts of the world are well documented, beginning in the 20th century (Wuebbles et al. 2017). The Midwestern USA (including Iowa) has been experiencing warmer and drier conditions in the 21st century (Wuebbles et al. 2017). Extreme summer weather may be more frequent in the future, possibly resulting in more high temperatures and drought events (Wuebbles et al. 2017), which may substantially impair honey bee foraging activities as plant communities are impacted. We suggest incorporating drought-tolerant plants in habitat conservation for improving pollinator diversity and honey bee health. Efforts to identify native plants that are drought tolerant and attractive to pollinators have resulted in 41 plants that can be potential choices for improving honey bee forage (Rowe et al. 2018). Our study suggests partridge pea, golden rod, and sunflower should be included as another climate-tolerant native forage plant

for honey bees. We suggest that further study is needed to better identify the best forage plants, given regional variation in flora and climate, that can provide honey bees with stable forage and nutrition in the context of a changing climate.

Conclusions

In conclusion, variation in land use did not induce differences in the abundance and diversity of plants used as sources of pollen by honey bees in the agricultural landscape of Iowa, USA. Instead, extreme weather events explained more of the variation in pollen forage collected by honey bees between years. Specifically, a combination of high temperatures and meteorological drought appeared to constrain bees' pollen foraging success. When considering restoring or reconstructing habitat to enhance the floral resources for honey bees, we recommend considering a plant community with species both favored by honey bees and also tolerant to extreme weather. Partridge pea is an example of a native plant that appeared to be both preferred by honey bees and have the capability to flourish during adverse weather. Such a plant could be an optimal choice for meeting broader conservation goals and supporting managed honey bee health.

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Tables and Figures

Table 1. Summary of information about research sites, colonies, and sampling frequency.

Year	Purpose [a]	Land use (site No.)	Colonies per site	Pollen traps per site [b]	Frequency of pollen sampling / colony inspection			
					Jun.	Jul.	Aug.	Sep.
2015	Weather	Soybean (n=10)	4	1		5/2	3/2	2/1
2016	Weather & Land use	Soybean (n=10)	4	1	5/2	4/2	3/2	1/1
2016	Land use	Prairie (n=5)	1	1	5/2	4/2	3/2	1/1
2016	Land use	DFV (n=5)	4	1	4/2	4/2	3/2	1/1
2017	Weather	Soybean (n=2)	2	2	1/1	2/1	2/2	3/2

[a] Research sites grouped for two research objectives, first to compare pollen abundance and diversity between land use types (soybean field, diverse fruit and vegetable [DFV] farm and prairie) in only 2016, and second to compare this pollen forage between years (2015-2017) at soybean fields.

[b] In 2015 and 2016, one of four colonies were chosen for installing an entrance pollen trap at each soybean field and diverse fruit and vegetable (DFV) farm. In 2016, the only one colony at each prairie was installed with an entrance pollen trap. In 2017, all of two colonies were installed with pollen traps at each soybean field.

Table 2. Percent of the ten most commonly represented plants in the bee-collected pollen from colonies kept at soybean fields, DFV farms and prairies during June-September of 2016.

Plant taxa [a]	Common name	% of each pollen (Mean ± SEM) by weight		
		Soybean	DFV	Prairie
Trifolium pretense	Red clover	36.90 ± 5.79	60.77 ± 12.72	23.50 ± 3.53
Triflolium repens	White clover	29.12 ± 4.25	10.36 ± 3.66	26.11 ± 11.29
Chamaecrista fasciculata	Partridge pea	8.27± 4.06	11.71 ± 10.51	26.14 ± 9.05
Lotus corniculatus	Birdsfoot trefoil	3.14 ± 2.11	2.46 ± 2.13	3.09 ± 1.35
Melilotus spp.	Sweet clover	2.91 ± 0.65	1.73 ± 0.37	1.29 ± 0.57
Ambrosia trifida	Giant Ragweed	2.90 ± 1.04	(0.7 ± 0.32)	(0.01 ± 0.01)
Saponaria officinalis	Bouncing bet	2.05 ± 1.82	0	0
Unknown species A		1.92 ± 1.68	0	0
Tilia Americana	Linden tree	1.88 ± 1.81	0	0
Zea mays	Corn	1.57 ± 0.75	(0.49 ± 0.48)	(0.02 ± 0.02)
Helianthus, Heliopsis & Siphilium spp.	Sunflower	(1.92 ± 1.68) [c]	2.64 ± 1.72	(0.24 ± 0.09)
Unknown species B		0	1.50 ± 0.72	0
Solidago spp.	Golden rod	(0.28 ± 0.22)	1.35 ± 0.56	1.43 ± 1.00
Daucus carota	Queen anne's lace	(0.23 ± 0.16)	1.00 ± 0.74	0

Table 2. Continued.

Plant taxa [a]	Common name	% of each pollen (Mean ± SEM) by weight		
		Soybean	DFV	Prairie
Unknown species C		0	0.98 ± 0.56	0
Iris versicolor	Northern blue flag	(1.04 ± 0.93)	(0.72±0.56)	7.23 ± 1.56
Dalea purpurea	Purple prairie clover	(0.72 ± 0.68)	(0.53 ± 0.34)	0.67 ± 0.41
Taraxacum officinale	Common dandelion	(0.2 ± 0.09)	(0.12 ± 0.1)	0.30 ± 0.30
Phlox paniculata	Garden phlox	(0.1 ± 0.07)	(0.02 ± 0.01)	0.27 ± 0.17
Total % of top 3 pollen [b]		74.29	82.84	75.75
Total % of top 10 pollen [c]		90.66	94.49	90.02

[a] Plant taxa was lay out at the order of high to low pollen abundance at soybean fields firstly and DFV farms secondly in 2016.

[b] Top 3 pollen include *T. pretense, T. repens and C. fasciculata* across three land use types .

[c] Each land use type has its unique set of 10 top pollen listed in the table.

[d] Values in parentheses indicated that pollen were not in the list of top 10 pollen.

Table 3. Percent of the ten most commonly represented plants in the bee-collected pollen from colonies kept at soybean fields across June-September 2015-2017.

Plant taxa [a]	Common name	% of each pollen type (Mean ± SEM)		
		2015	2016	2017
Triflolium repens	White clover	38.26 ± 9.09	29.12 ± 4.25	4.35 ± 1.78
Trifolium pratense	Red clover	28.53 ± 6.39	36.9 ± 5.79	5.25 ± 1.73
Chamaecrista fasciculata	Partridge pea	25.57 ± 6.97	8.27 ± 4.06	59.56 ± 2.32
Solidago spp.	Golden rod	2.6 ± 0.91	(0.28 ± 0.22)	19.43 ± 6.76
Circium spp.	Thistle	1.51 ± 0.73	(1.3 ± 0.81)	2.4 ± 2.16
Lotus corniculatus	Birdsfoot trefoil	0.51 ± 0.43	3.14 ± 2.11	0.9 ± 0.9
Helianthus, Heliopsis & Silphium spp.	Sunflower	0.45 ± 0.22	(0.1 ± 0.06)	3.51 ± 0.16
Sambucus spp.	Elder berry	0.3 ± 0.29	(0.02 ± 0.01)	(0.09 ± 0.04)
Ambrosia trifida	Giant ragweed	0.47 ± 0.2	2.9 ± 1.04	(0.14 ± 0.14)
Melilotus spp.	Sweet clover	0.48 ± 0.4	2.91 ± 0.65	(0.01 ± 0.01)
Saponaria officinalis	Bouncing bet	0	2.05 ± 1.82	0
Unknown species A		0	1.92 ± 1.68	0
Tilia americana	Linden tree	0	1.88 ± 1.81	0
Zea mays	Corn	(0.12 ± 0.09) [d]	1.57 ± 0.75	(0.45 ± 0.45)

Table 3. Continued.

Plant taxa [a]	Common name	% of each pollen type (Mean ± SEM)		
		2015	2016	2017
Taraxacum officinale	Common dandelion	0	(0.2 ± 0.09)	1.01 ± 1
Unknown species D		0	0	0.81 ± 0.81
Unknown species E		0	0	0.62 ± 0.62
Total % of top 3 plant [b]		92.35	74.29	84.24
Total % of top 10 plant [c]		98.67	90.66	97.84

[a] Plant taxa sorted by order of high to low pollen abundance in 2015, then 2016.

[b] Top 3 pollen include *T. pretense, T. Repens and C. fasciculate* in 2015 and 2016, *T. pretense, and C. fasciculata* and *Solidago* spp. in 2017.

[c] Each land use type has its unique set of 10 top pollen listed in the table.

[d] Values in parentheses indicated that pollen were not in the list of top 10 pollen.

Table 4. Regression analysis of monthly average pollen abundance with monthly GDD and rainfall using a linear mixed model.

Response variable	Month	Effect	Estimate	Standard Error	DF	t Value	Pr > |t|	Model AIC value
Pollen abundance	Jun	Intercept	-12.1457	7.8825	10	-1.54	0.1544	
		GDD	0.02842	0.01958	10	1.45	0.1773	40.6
	Jul	Intercept	8.1833	3.5325	16.6	2.32	0.0336	
		GDD	-0.02221	0.00888	16.5	-2.5	**0.0232**	54.8
	Aug	Intercept	2.1822	2.3736	7.95	0.92	0.385	
		GDD	-0.00825	0.006745	8.29	-1.22	0.255	69.6
	Sep	Intercept	-1.1188	11.1243	19	-0.1	0.9209	
		GDD	-0.00497	0.03637	19	-0.14	0.8927	88.2
Pollen abundance	Jun	Intercept	0.1689	0.7176	10	0.24	0.8186	
		Rainfall	-0.02268	0.01688	10	-1.34	0.2087	41.2
	Jul	Intercept	-2.4951	0.7135	14.6	-3.5	0.0034	
		Rainfall	0.01112	0.003991	20	2.79	**0.0114**	55.2
	Aug	Intercept	0.309	1.1555	12	0.27	0.7937	
		Rainfall	-0.00493	0.0057	9.98	-0.86	0.4076	70.4
	Sep	Intercept	-2.6735	0.9713	19	-2.75	0.0127	
		Rainfall	0.000239	0.00616	19	0.04	0.9694	91.8

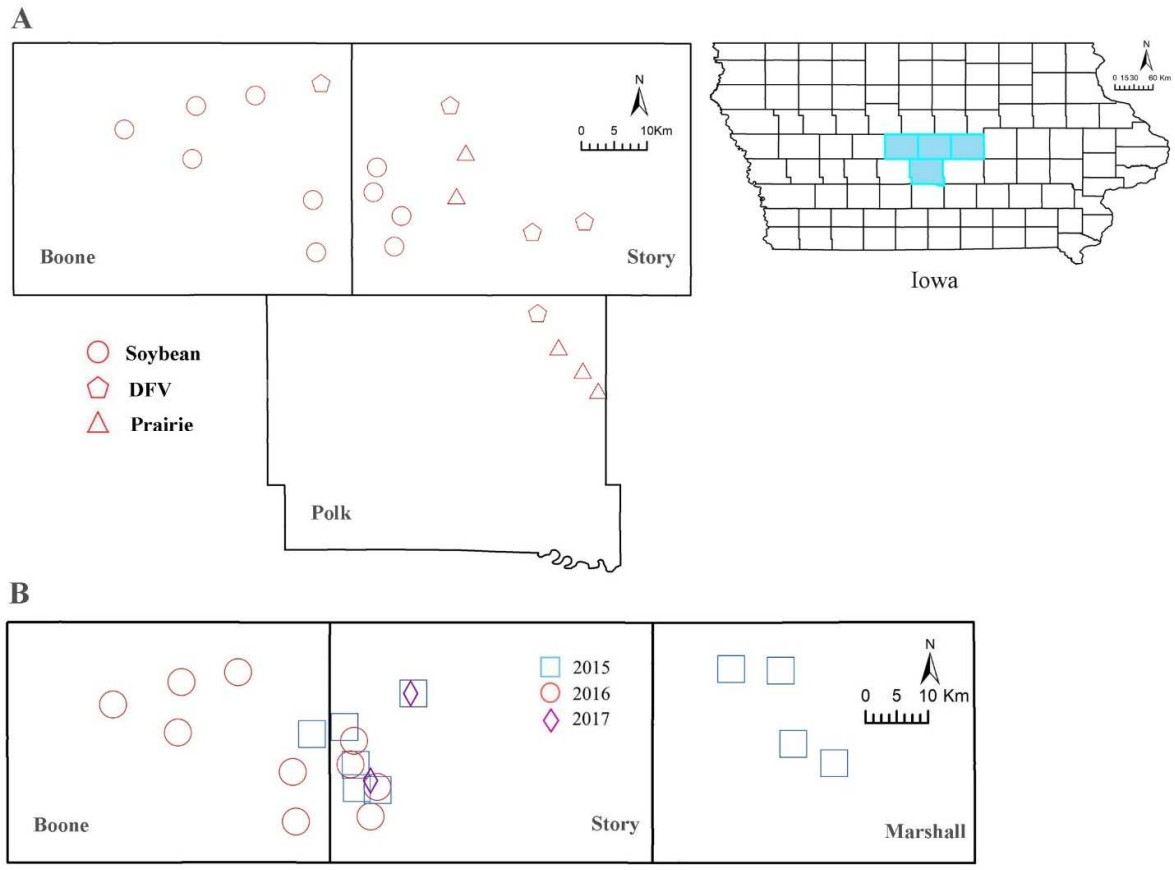

A
N
0 5 10Km
Boone
Story
Soybean
DFV
Prairie
Polk
N
0 15 30 60 Km
Iowa
B
2015
2016
2017
N
0 5 10 Km
Boone
Story
Marshall

Figure 1. Maps of research sites in Iowa for two research purposes. A) Research sites for comparing the pollen among three land uses (soybean field, DFV farm and prairie) in 2016 only. B) Research sites for comparing the pollen among years (2015-2017). Four counties, including Boone, Story, Polk and Marshall, are included in the map.

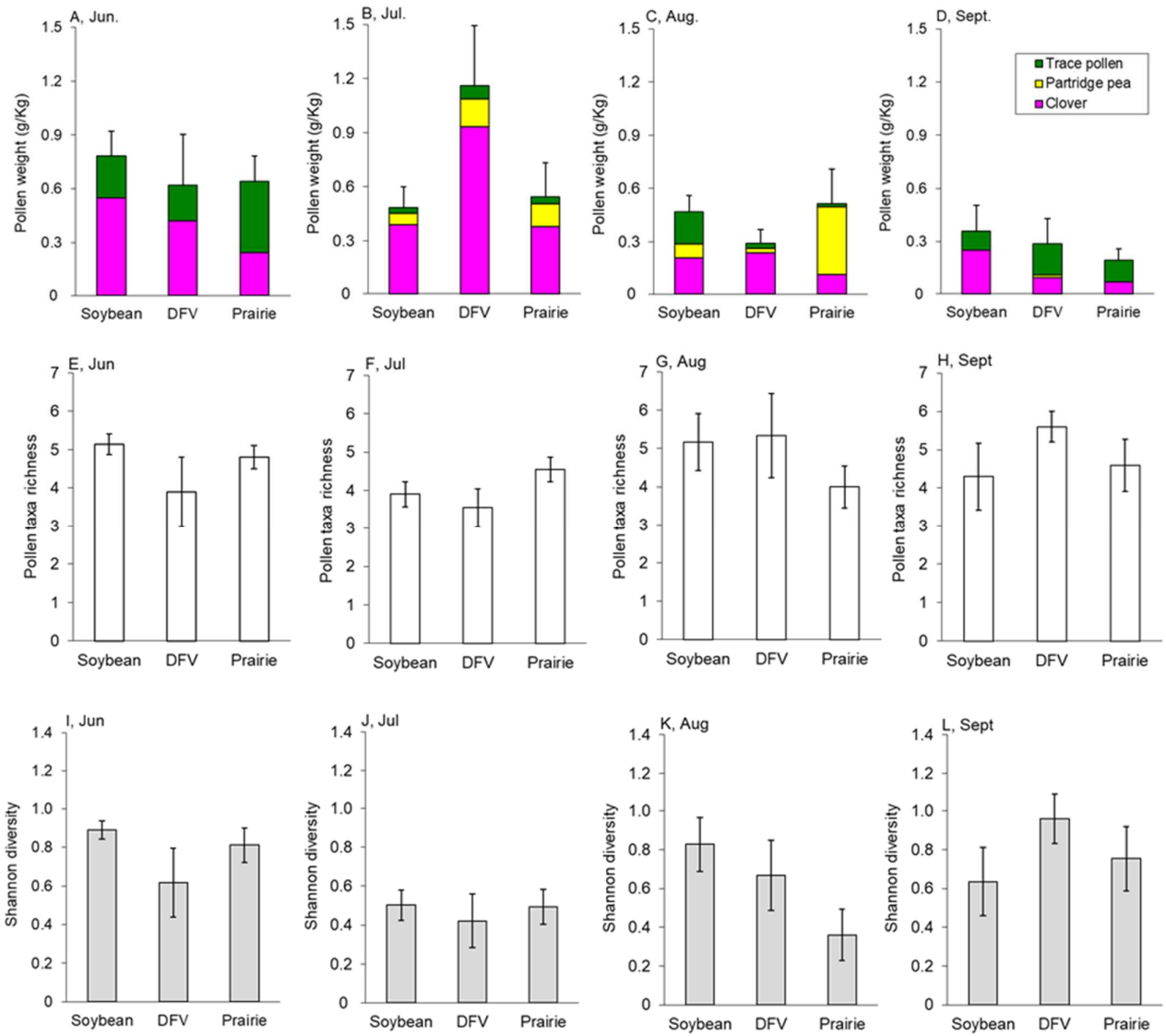

A, Jun.
B, Jul.
C, Aug.
D, Sept.
Pollen weight (g/Kg)
Trace pollen
Partridge pea
Clover
E, Jun
F, Jul
G, Aug
H, Sept
Pollen taxa richness
I, Jun
J, Jul
K, Aug
L, Sept
Shannon diversity
Soybean
DFV
Prairie

Figure 2. Comparisons of the monthly average pollen abundance (A-D), richness (E-H) and Shannon diversity (I-L) among land uses (soybean field, DFV farm, and prairie) using pollen data collected in 2016 only. The monthly average pollen weight (g) was normalized by monthly average colony weight (Kg). Error bars above colored column represented standard error of the average pollen in graphs A-D, taxa richness (E-H), and Shannon diversity (I-L). Different colors within each column indicated the three major pollen categories. Two categories were composed by common pollen sources that were > 5 % by weight, and the third categories was composed by any other pollen source that was < 5 %. ANOVA analysis indicated that pollen abundance, taxon richness and Shannon diversity was not significantly different among three land uses in any month ($P > 0.05$).

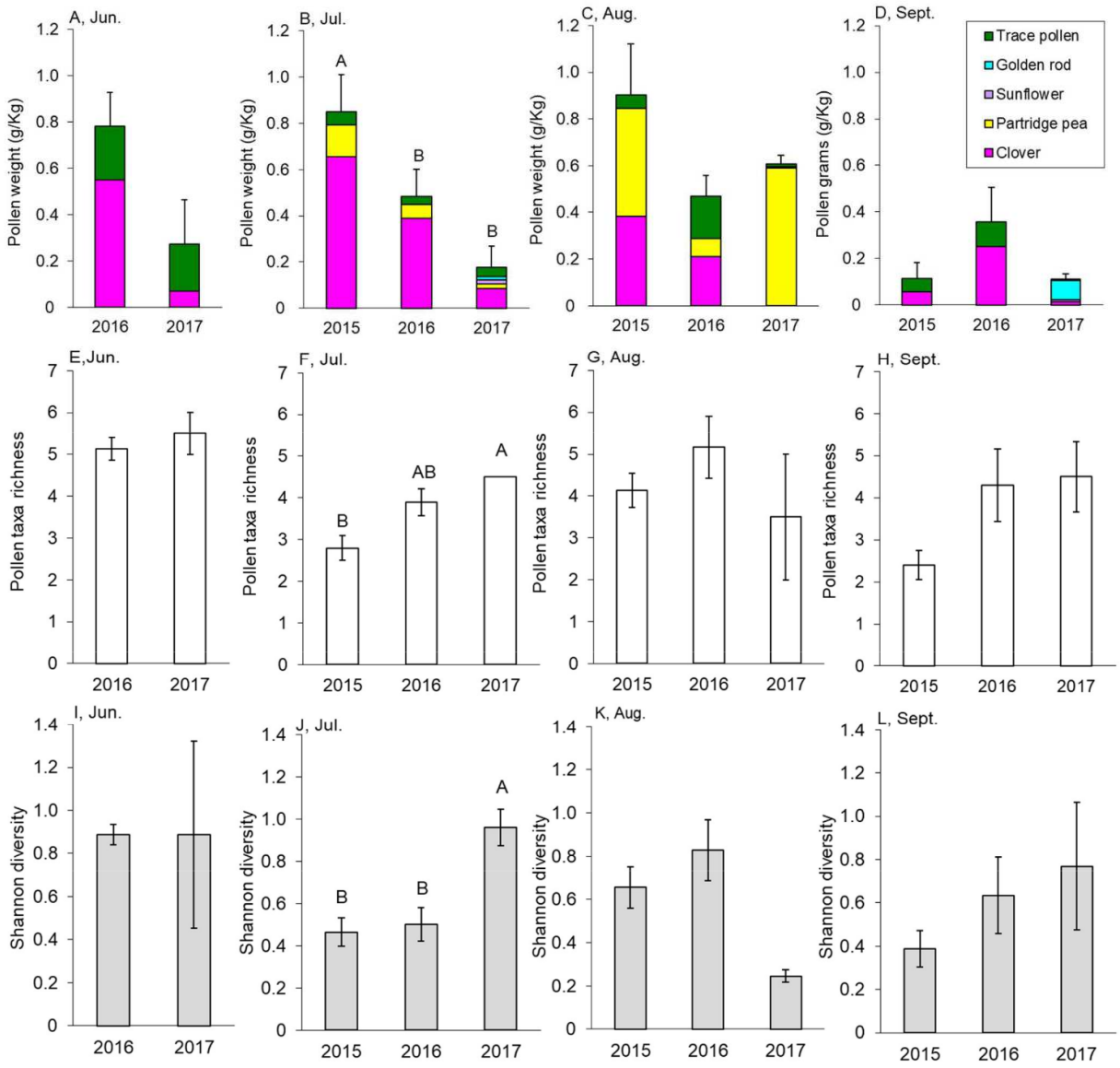

A, Jun.
B, Jul.
C, Aug.
D, Sept.
Pollen weight (g/Kg)
Pollen grams (g/Kg)
Trace pollen
Golden rod
Sunflower
Partridge pea
Clover
E,Jun.
F, Jul.
G, Aug.
H, Sept.
Pollen taxa richness
I, Jun.
J, Jul.
K, Aug.
L, Sept.
Shannon diversity
2015
2016
2017
A
AB
B

Figure 3. Comparisons of monthly average pollen abundance, taxon richness and Shannon diversity at soybean fields during 2015-2017. Different colors within each column indicated the four pollen categories that was > 5 % by weight, and one category that was composed by any other pollen source < 5 % by weight. Pollen abundance, taxon richness and Shannon diversity was significantly different in July (see analytical results in the main manuscript). In July, pollen abundance was lowest among three years and pollen taxon richness and the Shannon diversity index was highest among three years. Except for July, results of ANOVA indicated that pollen abundance, taxon richness and Shannon diversity was not significantly different among three years in any other months ($P > 0.05$).

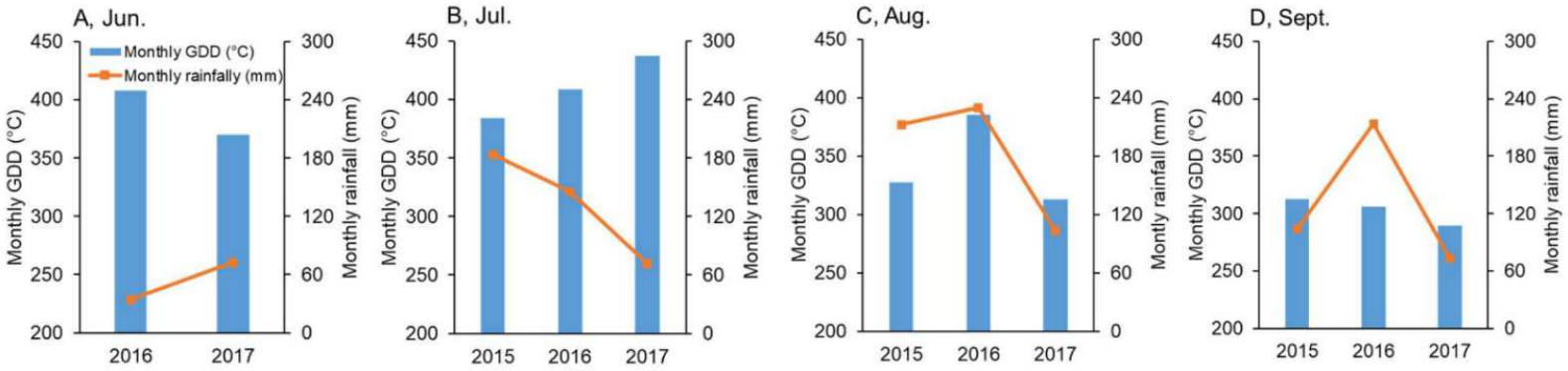


Figure 4. Means of monthly growing degree days (GDD) and rainfall in the study area in three years (2015-2017).

Supplementary Tables and Figures

Supp. Table 1. The upper limit of optimum monthly GDD and actual monthly GDD across three weather stations near our research sites and years.

County of weather stations	Month	Upper limit of optimum GDD/actual GDD in each year		
		2015	2016	2017
Story	Jun		**400.25 / 404.65**	378.85 / 370.2
	Jul	415.25 / 381.5	423.85 /400.8	**430.00 / 437.2**
	Aug	386.30 / 324.65	411.35 / 379.45	366.90 / 313.5
	Sept	356.55/303.5	356.10 / 293.45	336.85 / 289.55
Boone	Jun		**411.11 / 411.39**	
	Jul	426.67 / 391.39	437.50 / 416.94	
	Aug	399.17 / 338.89	427.78 / 391.67	
	Sept	371.39 / 326.11	216.94 / 366.94	
Marshall	Jun			
	Jul	418.50 / 378.5		
	Aug	386.95 / 318.95		
	Sept	362.40 / 307.95		
Average	Jun		**405.68 / 408.02**	378.85 / 370.2
	Jul	420.14 / 383.8	430.68 / 408.87	**430.00 / 437.2**
	Aug	390.81 / 327.5	419.56 / 385.56	366.90 / 313.5
	Sept	363.45 / 312.52	361.52 / 305.75	336.85 / 289.55

Supp. Table 2. Average historical rainfall (mm) of the three weather stations near our research sites measured in 1998-2017.

County	Jun	Jul	Aug	Sept
Boone	126.89	103.73	128.96	84.72
Marshall	120.74	85.19	101.85	70.56
Story	133.89	101.17	127.83	78.15
Average	127.17	96.7	119.54	77.81

Supp. Table 3. The flowering plant species found within 15 m distance from apiaries in three land uses.

Soybean field	DFV farm	Prairie
Asclepias spp.	*Asclepias* spp.	*Asclepias* spp.
Carduus nutans	*Carduus nutans*	
Daucus carota	*Daucus carota*	*Daucus carota*
Melilotus spp.	*Melilotus* spp.	*Melilotus* spp.
Pastinaca sativa	*Pastinaca sativa*	*Pastinaca sativa*
Penstemon digitalis		
Ratibida pinnata	*Ratibida pinnata*	*Ratibida pinnata*
Rudbeckia hirta	*Rudbeckia hirta*	*Rudbeckia hirta*
Taraxacum officinale		
Triflolium repens	*Triflolium repens*	*Triflolium repens*
Trifolium pratense	*Trifolium pratense*	*Trifolium pratense*
	Cirsium arvense	
	Cirsium vulgare	*Cirsium vulgare*
	Convolvulus spp.	
	Dalea purpurea	*Dalea purpurea*
	Erigeron strigosus	*Erigeron strigosus*
	Helianthus & silphium spp.	
	Heliopsis helianthoides	
	Lilium lancifolium	
	Lotus corniculatus	*Lotus corniculatus*
	Plantago lanceolata	
	Sambucus spp.	*Sambucus* spp.
	Solidago spp.	*Solidago* spp.
		Ambrosia artemisiifolia
		Echinacea purpurea
		Iris versicolor
		Monarda fistulosa

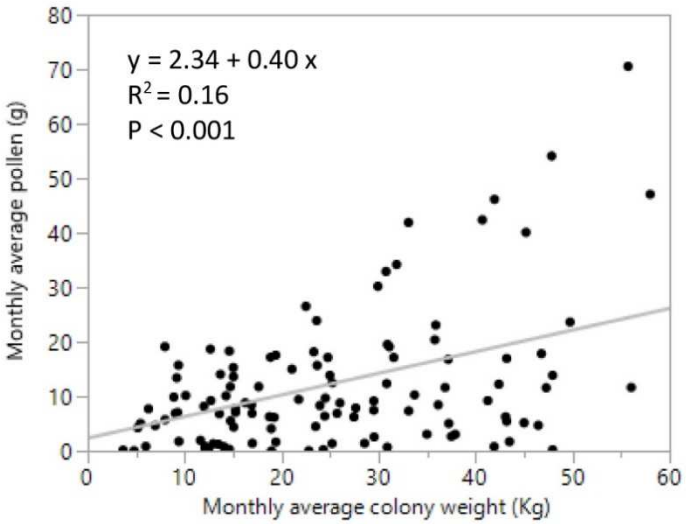
Supp. Table 3. Continued.

Soybean field	DFV farm	Prairie
		Pycnanthemum tenuifolium
		Silphium laciniatum
		Sisymbrium loeselii
		Verbena stricta
		Zizia aurea

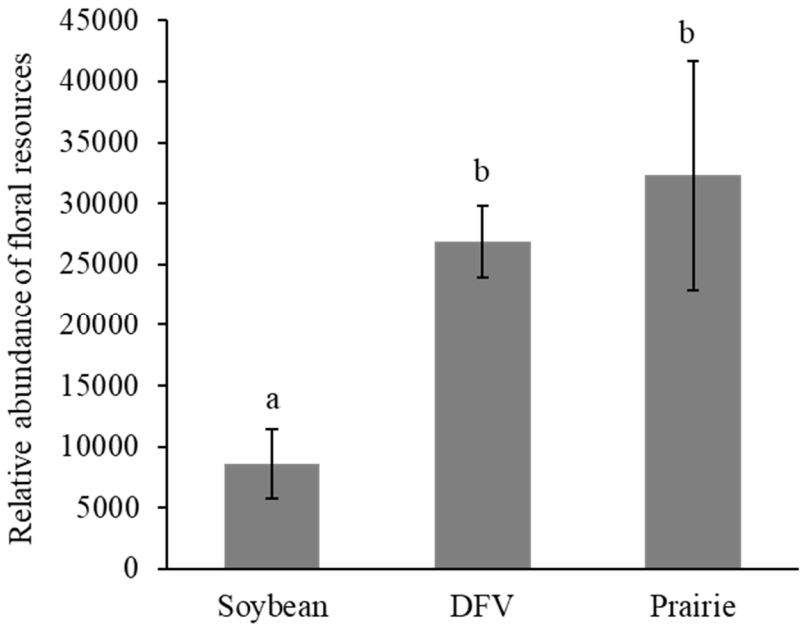
Supp. Table 4. Monthly average weight of clover (*Trifolium* spp.) pollen among years.

Month	Weight, g / Kg (Mean ± SEM)			D.F.	F value	p value
	2015	2016	2017			
June		0.55 ± 01.3 a	0.07 ± 0 b	1, 10	5.86	**0.036**
Jul.	0.66 ± 0.13 a*	0.39 ± 0.12 b	0.08 ± 0.05 c	2, 19	6.98	**0.005**
Aug.	0.38 ± 0.09 a	0.21 ± 0.05 b	0 c	2, 19	26.68	**< 0.0001**
Sept.	0.06 ± 0.04	0.25 ± 0.13	0.01 ± 0.01	2, 18	3.00	0.074

* Different letters beside mean values represent the results of multiple comparisons by Tukey's HSD.



Supp. Figure 1. Linear regression of the relationship between coloy weight and pollen amount collected by forager bees. Pollen amount collected by colony was positively correlated with colony weight ($P < 0.001$). Thus, we normalized monthly average pollen collection by montly average colony weight in this study.



Supp. Figure 2. Relative abundance of floral resources surrounding hives was determined by visually estimating flower abundance throughout the growing season during 2016. The number of flowers within a 15 m distance from our apiaries was assigned a category: 1-25, 26-200, 201-1000, 1001-5000 and >5000. The minimum value of each category (i.e., 1, 26, 201, 1001, and 5001) was used for estimating the relative abundance of floral resources near the apiaries located at three land use types. This assessment was taken every time when pollen was harvested from pollen traps in 2016, and we report the mean relative abundance (± SE). The statistical differences for the relative floral abundance of the entire season among land use types were analyzed with ANOVA using Tukey's HSD for multiple comparisons. Both DFV farms and prairies had significantly more floral resources than soybean fields, but there was no significant difference between DFV farms and prairies ($F = 19.23$, df = 2, 19, $P = 0.005$).

CHAPTER 4. NORTH AMERICAN PRAIRIE IS A SOURCE OF POLLEN FOR MANAGED HONEY BEES

Modified from a manuscript under review in Journal of Insect Science

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Abstract

Prairie was a dominant habitat within large portions of North America before European settlement. Conversion of prairies to farmland resulted into the loss of a large proportion of native floral resources, contributing to the decline of native pollinator populations. Efforts to reconstruct prairie could provide honey bees (*Apis mellifera*) a source of much needed forage, especially in regions dominated by crop production. To what extent honey bees, which were introduced to North America by European settlers, use plants native to prairies is unclear. We placed colonies with pollen traps within reconstructed prairies in central Iowa to determine if and when honey bees utilize plants found in prairie as a source of pollen. Honey bee colonies collected more pollen from nonnative than native plants during June and July. During August and September, honey bee colonies had more pollen from native plants that did not co-evolve with honey bees. This finding may be useful for addressing the nutritional health of honey bees, as colonies in this region frequently suffer from a dearth of forage contributing to colony declines during August and September, when crops and weedy plants cease blooming. These

results suggest that prairie can be a significant source of forage for honey bees in the later part of the growing season in the Midwestern USA; we discuss this insight in the context of honey bee health and biodiversity conservation.

Key words

Tallgrass prairie, habitat, beekeeping, foraging preference, landscape

Introduction

The tallgrass prairie biome of the Great Plains area of North America consists of many native plants that can support a diverse pollinator community (Tonietto et al. 2011, Smith et al. 2012, Robson 2014, Markiewicz 2016, Coleman 2019, Lamke 2019). The honey bee, *Apis mellifera*, was introduced to North America by European settlers and is a highly generalist forager known to take advantage of a wide variety of wild and cultivated flowering plant species. Although there are few hectares of prairie left due to widespread land conversion for urban and agricultural use in the Midwestern USA, some beekeepers target prairie or locations near prairie to install their apiaries (Werling et al. 2014, Otto et al. 2018) for the production of a honey crop (Nelson and Jay 1982). Honey bee colonies in agricultural landscapes used for soybean or corn production experience late season colony weight loss that can be rescued with late season access to reconstructed prairies, after crops and clovers have senesced (Dolezal et al. 2019). Because honey is the heaviest component of colony weight, this weight gain suggests that prairie is a source of nectar. Indeed, foraging honey bees have been observed to visit many native plants found in prairies (Tuell et al. 2014, Carr-Markell et al. 2020).

Honey bees have also been observed to collect pollen from plants in prairies (Carr-Markell et al. 2020), however, previous studies did not determine the abundance and diversity of pollen collected across the growing season. Although pollen is only a minor contributor to the weight of a colony, pollen is an important source of essential nutrients for colony and individual

honey bee growth (Wright et al. 2018). Determining which species of plants within prairies are a source of pollen and how much pollen is collected from prairies across the season could provide insight into how best to select native plants for habitat restoration that benefits both honey bees and native bees.

In addition to plants from habitats that are immediately adjacent to an apiary, honey bees also forage on plants widely distributed within the surrounding landscape, up to 13.5 km from their colony (Beekman and Ratnieks 2000). Thus, for colonies located near or within small islands of prairie in an agricultural or urban matrix, both native and nonnative plants have the potential to be a source of forage. Honey bees in North America have access to many nonnative plants, such as white clover (*Trifolium repens* L. [Fabales: Fabaceae]) (Sponsler et al. 2017), which is commonly used as forage in both their native (Europe) and nonnative (North America) ranges. To what extent honey bees use plants in prairies in North America, even when they are living in a wider landscape with nonnative resources present is unclear. In the current study, we address this question by providing a knowledge base of the species of plants used by honey bees across the growing season with apiaries placed in reconstructed North American prairies.

Our study was conducted in central Iowa, USA, a landscape containing small islands of prairie embedded within a matrix of farmland that is primarily committed to the production of corn and soybean (USDA-NASS 2019). The results of this study will provide useful insights into the utility of prairie for forage by the beekeeping industry.

Materials and Methods

Prairies and land cover of surrounding landscapes

We used two types of tallgrass prairies located in Iowa; isolated reconstructed tallgrass prairie and integrated reconstructed tallgrass prairie, as described in previous studies (Shepherd and Debinski 2005, Orlofske et al. 2011). In our study, isolated reconstructed prairie did not have

other prairies near them, but integrated reconstructed tallgrass prairie did. In Story County, we used two isolated reconstructed tallgrass prairies (named Meetz and Stargrass) to install our apiaries (S1 and S2) during 2016 and 2017 (Fig. 1, Supp. Table 1). In Polk county, we used three integrated reconstructed tallgrass prairies for our apiary locations (P1, P2 and P3) during 2016 and other three integrated reconstructed tallgrass prairies for our apiary locations (P4, P5 and P6) during 2018 (Fig. 1, Supp. Table 1). Those six integrated reconstructed prairies (P1-P6) were part of a conservation area located in Chichaqua Bottoms Greenbelt of Polk County in central Iowa. The integrated reconstructed prairies were larger than the isolated reconstructed prairie. These prairies were not mowed during the study period. One apiary was installed at each prairie during 2016-2018 resulting in five, two and three replications of apiaries/prairies in 2016, 2017 and 2018, respectively, with two prairies (Meetz and Stargrass) used in two continuous years (2016 and 2017) resulting into a total of eight prairies used in this study.

To help account for the potential impact the surrounding landscapes may have on honey bee foraging behavior, the percent of land cover types were measured using ArcGIS (Esri, Redlland, CA, USA). Although honey bees can forage up to 13.5 km away from their colony, most bees forage within a 1.6 km radius around the colony (Beekman and Ratnieks 2000, Carr-Markell et al. 2020), and the land cover within this buffer has been observed to influence honey bee health (Couvillon et al. 2014, Otto et al. 2016, Dolezal et al. 2019). Therefore, the percent of land cover types was measured within 1.6 km radius of each apiary. The land cover data layer was from USDA-NASS Cropscape (https://nassgeodata. gmu.edu/CropScape/). A total of 21 land cover types were identified and grouped into six major types for this study, including cropland, urban, grassland, woodland, wetland and vacant-land (Supp. Table 2).

Honey bee apiaries

All the colonies comprising apiaries installed in prairies were derived from managed stocks of Italian bees, *Apis mellifera ligustica*, first established at the Iowa State University (ISU) Research Apiary at the Horticulture Research Station in Ames, Iowa, USA, on 6 May 2016, 2 May 2017, and 6 June 2018. Colonies used in 2016 were initiated from "nucleus colonies" that contained frames with adult bees, immature bees (egg, larvae and pupae) and a honey bee queen in each colony. Colonies used in 2017 were initiated from "package bees" that were composed by adult bees and a honey bee queen held in wooden box with wire mesh made to conveniently deliver honey bees. The initial adult bee populations were similar across years (2016-2018) with approximately 7,000 adult bees per colony no matter how they were initiated. Colonies were housed in standard sized ("deep") Langstroth hive boxes with ten frames. The starting colony weights ranged between 5.41 kg to 9.91 kg in 2016, 6.52 to 8.79 kg in 2017, and 6.5 to 8.95 kg in 2018, when the wooden components (hive boxes, frames, bottom board and lid) were excluded. After the apiaries were established, no supplemental food was given to colonies.

All honey bee colonies were moved to prairies within three days after the colonies were established at the ISU apiary. The number of colonies in an apiary varied by year; one, two and four colonies were included in each apiary during 2016, 2017 and 2018, respectively. Frequency of apiary inspection varied by month and year; once in May and twice per month from June to August in 2016; once in June and October and twice per month from July to August in 2017; once in June, September and October, quadruple in July, twice in August in 2018. Any two inspections had an interval of at least seven days. Additional hive boxes were added if colonies lacked space to generate more brood or honey. Presence of the queen (i.e., visual confirmation of the queen, presence of eggs laid, or presence of young larvae less than three days old) was checked during each inspection. If the queen's presence was not observed, a new mated queen

was introduced within three days. To reduce infestations of Varroa mites (*Varroa destructor*), colonies were treated with a miticide (Apilife Var; Chemicals Laif SPA, Vigonza, Spain) once in August and September during 2016, and twice in September during 2017. During 2018, colonies were treated with Apiguard (Vita Europe Ltd, Valdosta, USA) once in August and September.

Pollen collection and identification

Pollen collected by honey bee foragers was harvested using hive entrance pollen traps (Brushy Mountain Bee Supply, Wilsonville, USA) placed on individual colonies. A plastic plate with star-shaped holes inserted into pollen traps pulled pollen pellets off the hind legs of foragers when they re-entered the hive. When pollen was not being collected, the plastic plate was removed from the trap so honey bees could leave and re-enter without being disturbed. The number of pollen traps at an apiary varied by year: one in 2016 and two in 2017 and 2018 (Supp. Table 1). Pollen was collected one to five times per month during a 24 h period without rainfall (frequency of collection summarized at Supp. Table 3). Across the season, we had 13, 5 and 7 pollen collections in 2016, 2017 and 2018 respectively. Due to variation in the number of apiaries and colonies within each apiary, those pollen collections resulted into 75, 16 and 21 pollen samples collected in this three year period.

After removing non-pollen debris, all pollen collections were weighed and stored at -20 °C. If an apiary had one pollen trap, 2 g pollen was extracted from the only pollen sample collected from that trap per 24 h period for pollen sorting and identification. If an apiary had two pollen traps, half of the pollen from each trap was mixed, 2 g pollen was extracted from the mixture, and the pellets were sorted by color. Pellets of the same color were weighed and mixed with Cablerla's fluid with fuchsin dye. The pollen solution was pasted on a glass side for taxonomic identification using a compound microscope. Morphological features of pollen were used to determine the plant species that produced this pollen. Pollen collected by honey bees was

compared to a reference pollen library created by extracting pollen from flowering plants (the plant taxa in the library referred in Supp. Table 4). The flowering plants in the reference pollen library were collected within 15 m from the apiaries that were placed at the prairies, as well as additional apiaries managed throughout Iowa as part of other experiments in 2015-2018 (Dolezal et al. 2019, Zhang et al. 2020). A total of 89 plant species were included in the reference pollen library comprised of 49 native and 40 nonnative plants (Supp. Table 4). Pollen collected by honey bees with morphological characteristics that did not correspond to specimens in the reference library were given a unique morphospecies identification (Supp. Table 4).

Statistical analysis

We used a standardized time period to determine if the diversity and amount of pollen collected by honey bees varied significantly across this three-year period. Data from one date per month were selected to compare diversity and abundance of pollen across months or years (Supp. Table 3). Because the frequency at which pollen was collected from the hive varied from one to five times per month (Supp. Table 3), a date within a month was selected that was as similar as possible to the dates in the same month for each year (Supp. Table 3). An apiary installed at a prairie was an experimental unit. The diversity (taxa richness) of pollen collected per apiary were compared among different months using a linear mixed effect model within the Proc Mixed function (SAS Institute, Cary, NC). This model also includes the effect of year and the interaction of month with prairie type (including isolated reconstructed prairie and integrated reconstructed prairie). The model did not include the interaction of year with prairie type due to a low amount of isolated reconstructed prairies used among years. Abundance of pollen (g) was compared among months using the same analysis described above.

The plants recognized as a source of pollen were grouped into two categories. We defined a plant as “native” if it is considered a component of prairies and was not introduced to

North America. We defined a plant as "nonnative" if it was introduced to North America or if it is not considered a component of North American prairies, e.g. a noxious weed. The designation of a plant to these two categories (native versus nonnative) was based on the Natural Resource Conservation Service (NRCS), United States Department of Agriculture (USDA) (https://plants.sc.egov.usda.gov/java/). The plant taxa richness represented in bee-collected pollen was the indicator of pollen diversity. Percent of pollen by mass was used as an indicator of relative abundance of pollen collected by honey bees either from native or nonnative plants. We compared the diversity and percent of pollen derived from native and nonnative plants using a linear mixed effect model within the Proc Mixed function (SAS Institute, Cary NC). Data from all collection dates were included in the statistical analysis. Response variables included plant taxa richness and percent of total pollen collected, and pollen category was an explanatory variable. In this mixed effect model, we also included the interaction of pollen categories (i.e., native or nonnative) with year or prairie type.

We conducted a linear regression analysis of plant taxa richness and percent of pollen derived from native and nonnative plants with six general land cover types to determine if any land cover in the surrounding landscapes explained the variation in diversity and abundance of pollen. The response variables were plant taxa richness and percent of pollen derived from native and nonnative plants, and the six land cover types were explanatory variables. We used a stepwise model selection to determine which land cover was most likely to be correlated with taxa richness and relative abundance of pollen derived from native or nonnative plants. Any land cover type meeting a 0.15 significance level was included in the model for further selection; while land cover categories not meeting a 0.15 significance level were removed from the model

selection process (Littell et al. 2002). The regression analysis was conducted for each month separately.

Results

Pollen diversity

A total of 57 plant taxa were found in the pollen traps, over three years from eight prairies. This community was composed of 12 native plants, 13 nonnative plants and 32 plants for which species could not be identified but were assigned a morphospecies name (Table 1, Supp. Table 5). Although these morphospecies were the most numerous category, they represented only 15% of the average mass of pollen collected throughout the sampling periods (Table 1). The average number of morphospecies was 4.75 (± 1), and the average number of identified species was 9.2 (± 0.8) from June to September of 2016-2018.

The most common native plants represented in our pollen traps (> 10 % by weight during any month across three years) were northern blue flag (*Iris versicolor* L. [Asparagales: Iridaceae]), purple prairie clover (*Dalea purpurea* Vent. [Fabales: Fabaceae]), common elderberry (*Sambucus Canadensis* L. (Dipsacales: Adoxaceae), partridge pea (*Chamaecrista fasciculata* [Michx.] Greene [Fabales: Fabaceae]), golden rod (*Salidago* spp. [Asterales: Asteraceae]), and sunflower (*Helianthus, Heliopsis & Silphium* spp. [Asterales: Asteraceae]). The most common identified nonnative plants represented in our pollen trap were white clover (*Trifolium repens* L. [Fabales: Fabaceae]), red clover (*Trifolium pratense* L. [Fabales: Fabaceae]), sweet clover (*Melilotus* spp. [Fabales: Fabaceae]) birdsfoot trefoil (*Lotus corniculatu*s L. [Fabales: Fabaceae]), and ragweed (*Ambrosia* spp. [Asterales: Asteraceae]) (Table 1). Despite being a common part of the central Iowa landscape, corn (*Zea mays* L. [Poales: Poaceae]) was only found in significant amount during one period (July of 2018) of this

three year study (Table 1). Soybean pollen was not present in trap collections, which is consistent with a similar study conducted in central Iowa (Dolezal el at. 2019, Zhang et al. 2020).

Taxa richness of plants used as pollen forage by honey bees did not differ across months ($F = 0.15$, df = 3, 30, $P = 0.93$) (Table 2), ranging from an average of five to six taxa per month (Fig. 2A). Taxa richness of plants used as pollen forage varied by year, with more plants used in 2016 than 2018 plants ($F = 4.34$, df = 2, 30, $P = 0.02$, multiple comparison with a Tukey-Kramer adjustment) (Table 2, Supp. Table 6). Taxa richness did not differ between prairie types (isolated versus integrated), nor was there a month by prairie type interaction (Table 2).

When plants were grouped as either native or nonnative, more nonnative taxa were collected by honey bees during June and July (June: $F = 43.81$; df = 1, 12, $P < 0.0001$. July: $F = 10.91$; df = 1, 12, $P = 0.0063$) (Fig. 2B, Table 3), but there was no difference during August and September (August: $F = 1.49$; df = 1, 12, $P = 0.245$; September: $F = 0.43$; df = 1, 12, $P = 0.5259$ (Fig 2B, Table 3).

Pollen abundance

The amount (g) of pollen collected by honey bees did not differ significantly among months ($F = 2.84$, df = 3, 30, $P = 0.0545$) (Fig. 3A) and the three years of this study ($F = 0.39$, df = 2, 30, $P = 0.6774$) (Table 2), but differ between prairie types ($F = 5.89$, df = 1, 30, $P = 0.0215$) (Table 2). The amount of pollen collected at integrated tallgrass prairies were larger than those at isolated tallgrass prairies ($F = 5.89$, df = 1, 30, $P = 0.0215$), but there was a month by prairie type interaction ($F = 2.92$, df = 3, 30, $P = 0.0499$) (Table 2). In June and September, more pollen was collected by colonies at integrated prairies than isolated prairies (June: $t = 2.39$, df = 30, $P = 0.0233$; September: $t = 3.25$, df = 30, $P = 0.0028$) (Supp. Table 7). In July and August, amount of pollen collected did not differ between isolated and integrated tallgrass prairies (July: $t = 0.18$, df = 30, $P = 0.8602$; August: $t = 0.32$, df = 30, $P = 0.7542$) (Supp. Table 7).

In June and July, the percent of pollen derived from nonnative plants was significantly greater than that from native plants (June: $F = 26.62$, df = 1, 12, $P = 0.0002$. July: $F = 9$, df = 1, 12, $P = 0.0111$) (Fig. 3B, Table 3). In August and September, the percent of pollen from native plants was significantly greater than that from nonnative plants (August: $F = 12.72$, df = 1, 12, $P = 0.0039$. September: $F = 5.41$, df = 1, 12, $P = 0.0384$) (Fig. 3B, Table 3).

Land cover and its relationship to collection of pollen from native and nonnative plants

Cropland was the most common land cover in the surrounding landscapes around apiaries located in prairies (Fig. 4, Supp. Table 2). Grassland was the second most common land cover; this measure included the area of prairie where we installed apiaries (Fig. 4, Supp. Table 2).

Regression analysis was used to determine if variation in the landscape surrounding the apiaries explained variation in the diversity and abundance of native and nonnative plants represented in the pollen collected by the colonies. We anticipated a positive relationship between these parameters with pollen from native plants and the amount of grassland surrounding the colonies. Overall, grassland cover did not explain any variation in the diversity or abundance of native pollen collected by these colonies (Table 4). In September, woodland and urban land cover were positively correlated with diversity of native plants represented in pollen (Table 4). Percent of urban land and woodland in the surrounding landscapes was positively correlated with percent of native plants represented in pollen in June and July, respectively (Table 4).

We anticipated that abundance and diversity of pollen from nonnative plants would be positively correlated with urban and cropland cover. Overall, the regression analysis supported this prediction, with increases in the abundance and diversity of nonnative pollen associated with greater percent of urban or cropland cover in certain months. For example, in June, both diversity and percent of nonnative plants represented in pollen collected by honey bees was positively

associated with urban land cover (Table 5). In September, percent of nonnative plants represented in pollen was positively associated with cropland. Grassland and woodland were negatively associated with diversity of nonnative plants during June and July, respectively (Table 5). In July, wetland cover had a negative association with percent of pollen derived from nonnative plants (Table 5).

Discussion

Honey bees are a globally-distributed, semi-domesticated insect with a polyphagous feeding range (Kaluza et al. 2017). Although honey bees are known to forage on a wide variety of crops, weeds, as well as native species in many regions (Sponsler et al. 2017), the extent to which they use native versus nonnative plants outside of their original range is not well-understood. We provide a detailed assessment of how honey bees used native and nonnative plants for pollen across the growing season, in the context of reconstructed tallgrass prairies in the Midwestern US, a critical area for pollinator conservation as well as bee health (Grixti et al. 2009, Zaya et al. 2017). Our results suggest honey bees use native plants in prairies throughout the season, even though honey bees did not co-evolve with native Midwestern USA prairie plants. This finding confirms that honey bees as generalist foragers can adapt to versatile habitats within their introduced range. Honey bees utilized more nonnative plants in the early season, but used more native prairie plants in the late season. This suggests that native habitats may provide an especially important source of pollen to honey bees in the late season, a time of forage dearth observed in central Iowa (Dolezal et al. 2019).

Overall, 12 native plant taxa were identified in the pollen collected by honey bees from Iowa prairies. Our visual inspection of flowering plants found adjacent to colonies revealed the presence of nine of those taxa (except *Oenothera biennis* L. [Myrtales: Onagraceae], *Sambucus Canadensis* L. [Dipsacales: Adoxaceae] and *Tilia Americana* L. [Malvales: Malvaceae]),

suggesting that honey bees utilized the prairies for pollen. Pollen from native plants were found in pollen traps across the entire season, with different species represented at varying times. For example, northern blue flag (*I. versicolor*) and purple prairie clover (*D. purpurea*) were collected during June and July, and partridge pea (*C. fasciculata*), golden rod (*Salidago* spp.) and sunflowers (*Helianthus* spp.) were collected during August and September. These time periods overlap with the flowering phenology of these taxa (Henry 2002, USDA-NRCS 2002, 2003, Carr 2009, Pavek 2011, Houck and Row 2019). Honey bees are likely using prairie plants depending upon both their flowering phenology also the flowering of nonnative species.

Honey bees frequently used nonnative plants throughout the season. Honey bees may have found these nonnative plants within the prairies, but more likely they were found in landscape features such as crop fields, field margins, and roadsides. For example, a significant amount of pollen from corn was found in traps during July of 2018, likely from surrounding corn fields that were in anthesis. The diversity and abundance of pollen in relation to variation in the surrounding landscape suggested that urban and crop landscapes may be a source of pollen derived from nonnative plants. The pattern of using both native and nonnative plants as a source of pollen suggested honey bees are modifying their foraging behavior based on the availability of flowering of plants within different features of their overall foraging landscape. Our observations and analysis were consistent with a recent study that analyzed the dance language of honey bee foragers and compositions of pollen collected by them that indicate a simultaneous use of prairies and other land covers in Midwestern US landscapes (Carr-Markell et al. 2020).

The balance of pollen from native or nonnative plants varied significantly by month. During June and July, honey bees used more nonnative plant taxa (Fig. 2) and collected more pollen from them than native plants (Fig. 3). During August and September, the number of native

and nonnative plants in bee-collected pollen did not differ, but more pollen was collected from native plants. This pattern suggests that as nonnative plants stop blooming, honey bees switch to native plants blooming later in the growing season. The early predominance of nonnative plants represented in bee-collected pollen may be due to greater attraction or availability of pollen from nonnative plants. Nonnative clover species such as white clover, red clover, and sweet clover are commonly found in field edges and roadsides in this study region. Both honey bees and these nonnative plants originated from Europe, and may have a co-evolutionary history to make honey bees prefer these plants in North America. For example, the length of the flower tubes for these plants are shorter or equal to the extended proboscis of honey bees, making it easier for foragers to reach the nectar or pollen in the flower (Alexandersson and Johnson 2002). Foraging preference on those nonnative plants could also be related to high nutritional value of their pollen (Rayner and Langridge 1985, Russo et al. 2019). Many nonnative plants that successfully colonize outside their native habitats tend to flourish in disturbed habitats such as field edges, roadsides, and urban lands of a new region by taking advantages of an ecological niche, and have an adaption strategy of blooming early for a successful reproduction (Grime 2006, Colautti and Barrett 2013). This characteristic provides an opportunity for honey bees to collect more pollen from nonnative plants. For example, nonnative clover such as white clover (*Trifolium repens*) starts blooming during the early part of the growing season, and as noted by Dolezal et al. (2019) clover bloom declines in August, which may facilitate a switch to more abundant native plants found in prairies. Plant surveys conducted in the prairies used in this study revealed a diverse community of native plants that flower throughout a growing season, including August and September (Shepherd and Debinski 2005, Ohnesorg 2008, Orlofske et al. 2011, Summerville et al. 2011, Delaney et al. 2015).

Prairie plants may also provide a source of nectar for honey bees in this landscape after other common plants in the Midwest cease blooming. Honey bees kept adjacent to commercial soybean fields in central Iowa suffered colony weight loss beginning in August (Dolezal et al. 2019). Colony weight peaked in August, followed by a steep decline that appeared to coincide with forage dearth. This weight loss was reversed by giving honey bees access to reconstructed prairies (Dolezal et al. 2019), which contain numerous native plants that flower after August and may provide important sources of late season nectar (e.g. goldenrod). Extending the results of Dolezal et al. 2019, our results suggest that access to prairies could also help honey bees avoid a shortage of pollen later in the season. Late season pollen may provide an important source of protein and lipids that can enhance fat body growth for "winter bee" workers that will need extra nutrient stores to survive the winter (Döke et al. 2015). Improvements to the abundance and diversity of pollen consumed by honey bees can result in improved survival of adult honey bees when exposed to viral pathogens (Dolezal et al. 2019, Zhang et al. 2020). Future experiments should consider if the pollen derived from prairie plants directly benefit the health of honey bees.

Pollen collected by colonies in integrated tallgrass prairie was more abundant than that collected in isolated tallgrass prairies. This may be due to colonies located in integrated tallgrass prairies having greater access to native forage than these placed in isolated prairies, first, because of larger area of each integrated prairie, and second, because of adjacency to other surrounding prairies. In contrast, colonies kept in isolated tallgrass prairies may lack sources of native forage in the surrounding landscapes. Future studies should consider aspects of the foraging response of honey bees to the varying size of prairies. We did not survey the prairie plant community and the future studies should consider honey bee' response to plant community of different compositions in prairie.

In conclusion, we observed that when apiaries were placed in Midwestern tallgrass prairies, honey bees used many members of the plant community that are native to North America. Although cultivated areas can be an important source of pollen in June and July (especially nonnative species such as clover), prairies became a more important source of pollen in August and September. The native plants such as partridge pea and golden rod could buffer late season colony decline when floral resources in cultivated areas have declined steeply. If a habitat is created to benefit honey bees, increasing the diversity of native plants that are used as a source of forage by honey bees should be considered. It has been suggested that conservation for honey bees focus on a simpler seed mix of plants attractive to honey bees including primarily composed with two nononative species (*Melilotus* and *Medicago*) and one native species (*Linum*) (Otto et al. 2017). Although these plants may be preferred by honey bees, they mainly flower in the early part of the growing season. Our results suggest that honey bees use of native plants may depend upon the seasonality of both native and nonnative plants present in the landscape. Planting a more diverse mixture of forbs, especially in regions which experience precipitous declines in floral resources, can support honey bees as well as wild pollinators. If the goal is to benefit both, it may be possible to limit potential competition by selecting a mixture of plants that contain preferred sources for honey bees, as well as some species that are more preferred by wild bees. Overall, these results suggest native prairie restoration may be a conservation management strategy that can provide benefits to managed honey bees, while also benefiting native biodiversity.

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Tables and Figures

Table 1. Average (± SE) percent of pollen derived from native and nonnative plants in each month of 2016-2018.

Pollen taxa	2016 (n = 5)				2017 (n =2)				2018 (n = 3)			
	Jun	Jul	Aug	Sep	Jun	Jul	Aug	Sep	Jun	Jul	Aug	Sep
*Chamaecrista fasciculata**	0	10.01 ± 9.97	69.94 ± 17.76	0	0	0	79.94 ± 17.73	0	0	0.75 ± 0.75	61.34 ± 7.74	19.28 ± 15.81
*Dalea purpurea**	0.02 ± 0.02	2.38 ± 1.70	0	0	0	30.61 ± 30.32	0	0	4.25 ± 4.25	0.56 ± 0.41	0	0
Echinacea spp.*	0	0	0	0	0	0	0	0	0	0.07 ± 0.07	0	0
*Eryngium yuccifolium**	0	0	0	0	0	0	0	0	0	0.45 ± 0.45	0	0
Helianthus, Heliopsis & Silphium spp.*	0	0	0.47 ± 0.43	0	0.71 ± 0.71	1.28 ± 1.28	0.37 ± 0.37	12.80 ± 8.63	0	9.00 ± 8.77	0	0.62 ± 0.62
*Iris versicolor **	25.01 ± 6.17	0.04 ± 0.04	0	3.82 ± 2.12	0	0	0	0	34.00 ± 8.11	0.42 ± 0.42	0	0
*Monarda fistulosa & Pycnanthemum virginianum**	0	0.22 ± 0.22	0.05 ± 0.05	0	0	2.02 ± 2.02	0.26 ± 0.15	0.09 ± 0.09	0	2.47 ± 2.36	0	0
*Oenothera biennis**	0	0	0	0	0	0	0	0	0	0.07 ± 0.07	0.64 ± 0.64	0
Phlox spp.*	0	0	0	6.40 ± 4.60	0	2.78 ± 2.78	0	0.07 ± 0.07	0	0	0.99 ± 0.15	0
*Sambucus Canadensis**	0.02 ± 0.02	0	0	0	12.76 ± 5.72	0.54 ± 0.54	0	0	0.97 ± 0.35	0	0	0
Solidago spp.*	0	0	0.06 ± 0.05	26.92 ± 14.21	0	0	0.38 ± 0.38	80.58 ± 12.12	0	0	1.11 ± 1.11	36.15 ± 20.37
*Tilia Americana**	0	0	0	0	0	0	0	0	1.33 ± 1.33	0	0	0
Ambrosia spp.*,§	0	0	0.0 5± 0.04	0	0	0	10.56 ± 10.56	0.09 ± 0.09	0	0	0	0
*Chenopodium album**,§	0	0	0	0	0	0	0	0	0	0	1.31 ± 0.66	1.45 ± 0.76
Cichorium intybus §	0	0	0	0	0	0	0	0	0	0.28 ± 0.28	0	0
Cirsium spp. §	0.04 ± 0.02	0.09 ± 0.06	0.36 ± 0.27	0	6.63 ± 5.05	4.46 ± 4.4	0.41 ± 0.12	0	4.18 ± 3.17	0	0	0
Daucus carota §	0	0	0	0	0	0	0	0	0	1.39 ± 1.39	0	0

Pollen taxa	2016 (n = 5)				2017 (n =2)				2018 (n = 3)			
	Jun	Jul	Aug	Sep	Jun	Jul	Aug	Sep	Jun	Jul	Aug	Sep
Lotus corniculatus §	6.25 ± 2.54	2.32 ± 1.43	0.4 ± 0.23	0	17.79 ± 17.79	0.66 ± 0.66	0	0	4.37 ± 2.19	0.47 ± 0.40	0.98 ± 0.98	0
Melilotus spp. §	2.85 ± 1.41	0.51 ± 0.21	0.2 5± 0.25	0	0	0	0.10 ± 0.10	1.22 ± 1.22	29.46 ± 10.08	4.64 ± 1.98	0	0
Pastinaca sativa §	0	0.44 ± 0.24	0.10 ± 0.10	0	6.45 ± 6.45	0	0	0	0	0	0	0
Phaseolus vulgaris	0.01 ± 0.01	0	0	0	0	0	0	0	0	0	0	0
Taraxacum officinale §	0	0	0	3.64 ± 3.60	0	0.12 ± 0.12	0.47 ± 0.47	1.32 ± 0.33	0	0	0	0
Trifolium pratense	10.2 ± 7.79	52.3 ± 12.59	12.96 ± 8.02	24.58 ± 17.40	1.24 ± 1.24	8.08 ± 6.97	6.78 ± 6.34	0	0	2.91 ± 2.54	0	0.74 ± 0.74
Trifolium repens	32.63 ± 7.28	30.63 ± 13.47	14.45 ± 13.97	31.69 ± 19.21	54.42 ± 0.04	3.41 ± 2.89	0.09 ± 0.09	1.50 ± 0.23	13.89 ± 0.91	7.32 ± 4.48	2.46 ± 2.13	0
Zea mays	0.03 ± 0.03	0.02 ± 0.02	0	0	0	0	0.17 ± 0.17	0	0	46.72 ± 9.41	0	0
Total of unidentified pollen taxa [a]	22.93 ± 6.39	1.02 ± 0.63	0.89 ± 0.56	2.93 ± 1.29	0	46.05 ± 44.64	0.46 ± 0.46	2.34 ± 2.23	7.55 ± 2.46	22.45 ± 14.44	31.16 ± 5.41	41.76 ± 26.45

* Native plants as pollen source. § Invasive plants as pollen source. [a] Each unidentified pollen taxa was record separately and designated by a morphospecies name (refer to Supp. Table 6).

Table 2. Analysis of the variation in diversity (taxa richness) and abundance of pollen (gram) collected by month (June to September), year (2016-2018) and prairie type (isolated versus integrated) using linear mixed effect models.

Pollen	Effect	df	F value	P value
Diversity	Year	2, 30	4.34	**0.0222**
	Month	3, 30	0.15	0.9257
	Prairie type	1, 30	0.43	0.5162
	Month × Prairie type	3, 30	0.48	0.6973
Abundance	Year	2, 30	0.39	0.6774
	Month	3, 30	2.84	0.0545
	Prairie type	1, 30	5.89	**0.0215**
	Month × Prairie type	3, 30	2.92	**0.0499**

Table 3. Comparisons of taxa richness and percent of pollen derived from two pollen categories (native and nonnative plants) and interaction of pollen category with year or prairie type using linear mixed effect models.

Pollen	Month	Effect	df	F value	P value
Taxa richness	June	Pollen category	1, 12	43.81	**<0.0001**
		Prairie type × Pollen category	2, 12	2.08	0.1677
		Year × Pollen category	4, 12	2.8	0.0746
	July	Pollen category	1, 12	10.91	**0.0063**
		Prairie type × Pollen category	2, 12	0.36	0.7064
		Year × Pollen category	4, 12	1.16	0.3768
	August	Pollen category	1, 12	1.49	0.245
		Prairie type × Pollen category	2, 12	6.03	0.0154
		Year × Pollen category	4, 12	2.22	0.1282
	September	Pollen category	1, 12	0.43	0.5259
		Prairie type × Pollen category	2, 12	3.47	0.0648
		Year × Pollen category	4, 12	3.58	0.0382
Percent	June	Pollen category	1, 12	26.62	**0.0002**
		Prairie type × Pollen category	2, 12	1.09	0.3666
		Year × Pollen category	4, 12	1.06	0.4174
	July	Pollen category	1, 12	9	**0.0111**
		Prairie type × Pollen category	2, 12	0.32	0.733
		Year × Pollen category	4, 12	3.13	0.0559
	August	Pollen category	1, 12	12.72	**0.0039**
		Prairie type × Pollen category	2, 12	1.41	0.2821
		Year × Pollen category	4, 12	0.68	0.6194
	September	Pollen category	1, 12	5.41	**0.0384**
		Prairie type × Pollen category	2, 12	3.16	0.0789
		Year × Pollen category	4, 12	3.77	0.0327

Table 4. Regression of taxa richness and percent of pollen derived from native plants, considering land cover type surrounding our apiaries within 1.6 km radius.

Pollen from native plants [a]	Month	Land cover	Slope	Standard error	F value	P value	Model R^2
Taxa richness	June	Grassland	0.02719	0.01320	4.24	0.0733	0.3467
	July	Woodland	0.28657	0.1497	3.66	0.0919	0.3142
	August	Urban	-0.11301	0.04921	5.27	0.0507	0.3973
	September	Urban	0.09748	0.03361	8.41	**0.023**	0.5020
		Woodland	0.2536	0.06043	17.61	**0.0041**	0.7738
Percent	June	Urban	2.1965	0.77688	7.99	**0.0222**	0.4998
	July	Woodland	6.68393	2.17626	9.43	**0.0180**	0.5150
		Vacantland	-16.85405	9.91103	2.89	0.1328	0.6568
	August	N/A [b]					
	September	Woodland	9.48501	4.81895	3.87	0.0846	0.3263

[a] Percent and taxa richness of pollen were response variables and six general land covers were explanatory variables. [b] N/A, data did not pass the model selection for exploring a significant relationship.

Table 5. Regression of taxa richness and percent of pollen derived from nonnative plants, considering land cover type surrounding our apiaries within 1.6 km radius.

Pollen from nonnative plants [a]	Month	Land cover	Slope	Standard error	F value	P value	R^2
Taxa richness	June	Urban	0.15233	0.02447	38.76	**0.0004**	0.7502
		Grassland	-0.01871	0.00674	7.71	**0.0274**	0.8811
	July	Woodland	-0.28665	0.11867	5.83	**0.0421**	0.4217
	August			N/A [b]			
	September	Wetland	0.11613	0.05302	4.80	0.0599	0.3749
Percent	June	Urban	3.43787	1.42303	5.84	**0.0464**	0.2933
		Woodland	4.09145	2.48372	2.71	0.1435	0.4907
	July	Wetland	-4.64442	1.32319	12.32	**0.008**	0.6063
	August	Grassland	-0.89201	0.52412	2.90	0.1272	0.2658
	September	Cropland	1.44687	0.43876	10.87	**0.0109**	0.5761

[a] Percent and taxa richness of pollen were response variables and six general land covers were explanatory variables. [b] N/A, data did not pass the model selection for exploring a significant relationship.

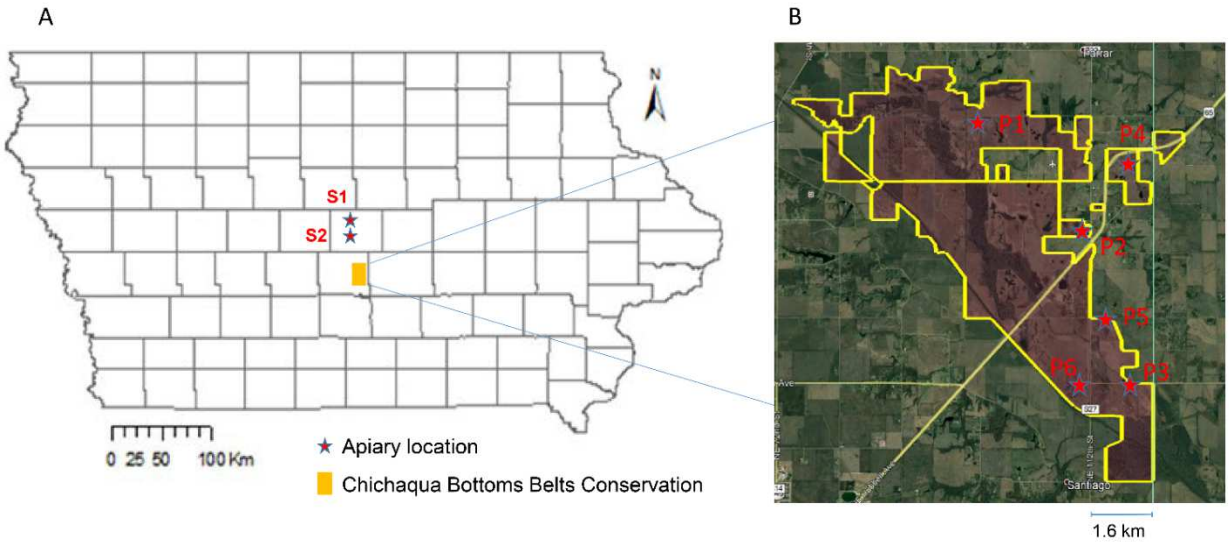

Figure 1. Location of apiaries. A) Map of Iowa with the locations of apiaries S1 and S2 used in both 2016 and 2017, and the location of Chichaqua Bottoms Greenbelt Conservation Area where apiaries were installed in 2016 and 2018. B) The outline map of Chichaqua Bottoms Greenbelt Conservation Area with the apiaries marked with star-shaped symbols. Apiaries P1-P3 were used in 2016 and P4-P6 used in 2018. The outline of Chichaqua Bottoms Greenbelt Conservation Area was provided by Doug Sheeley, Polk County (Iowa) Conservation.

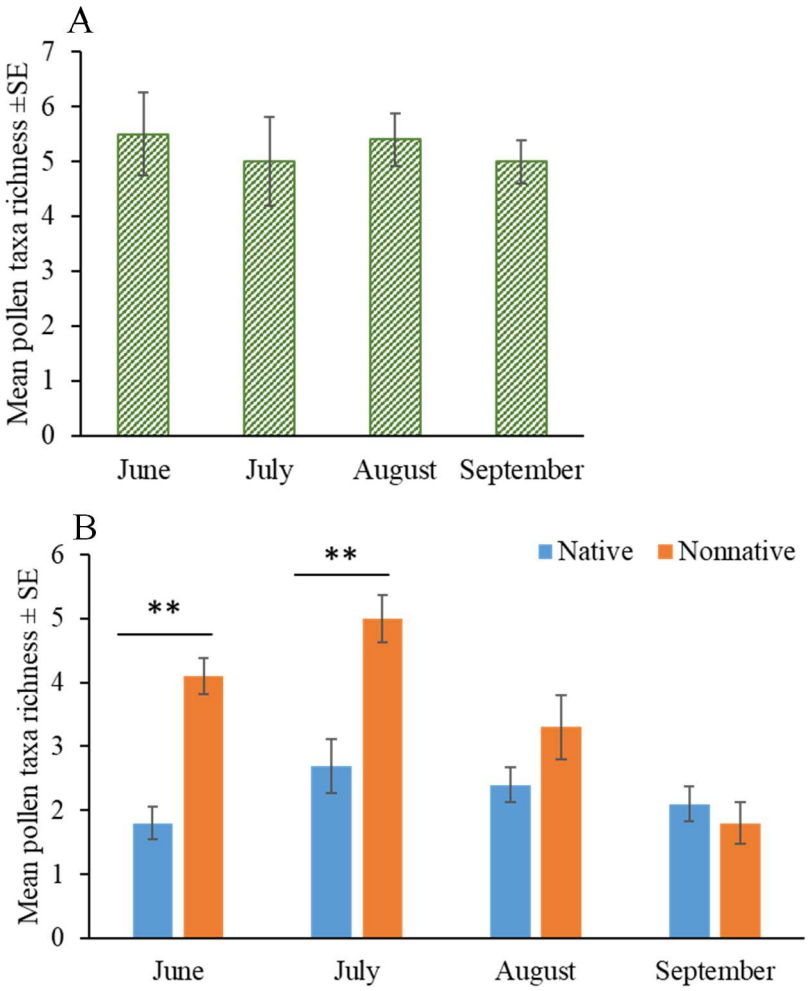

Figure 2. Diversity of pollen across the growing season (A) and comparison of diversity of pollen derived from native versus nonnative plants. Bars on each column represented the standard error. A) One pollen collection date in each month was selected for comparing the diversity of pollen across months. Diversity of pollen was not significantly different among months ($F = 0.15$, df = 3, 30, $P = 0.9257$). B) Data from all the pollen collection dates were used for comparing diversity of pollen derived from native versus nonnative plants. The statistical analysis is summarized in Table 3. ** $P < 0.01$.

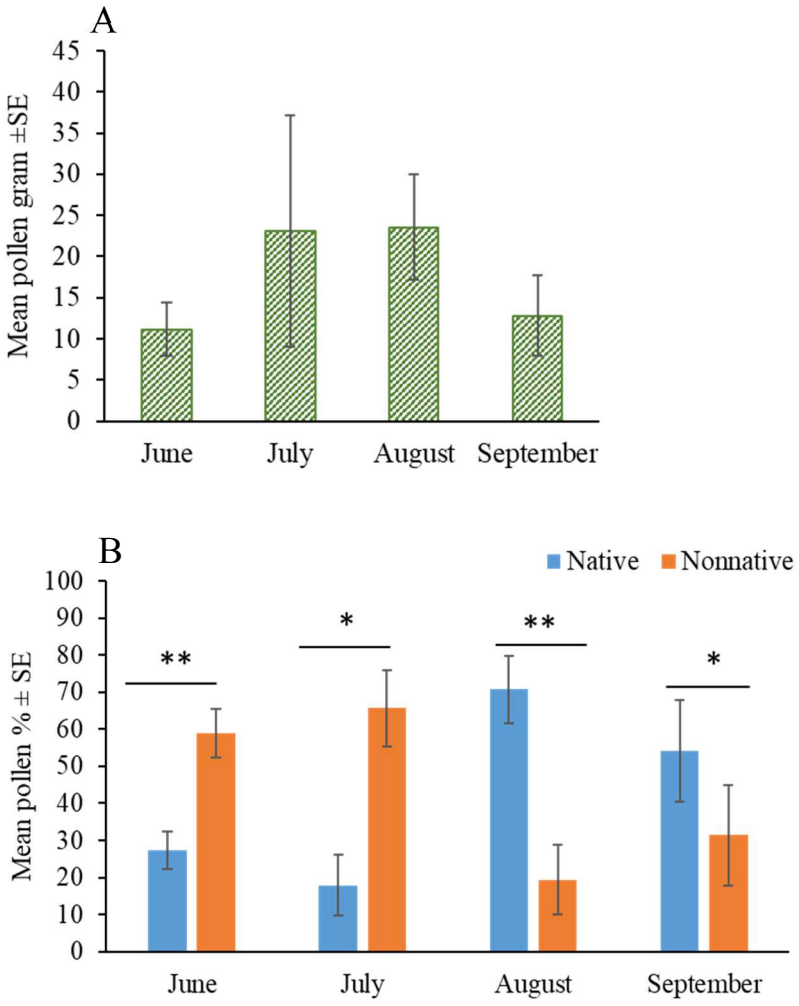

Figure 3. Pollen abundance (g) across the growing season (A) and comparison of percent of pollen by weight derived from native versus nonnative plants (B). A) One pollen collection date in each month was selected for comparing the abundance of pollen across months Data from all the pollen collection dates were used for the analysis. Amount of pollen was not significantly different among months ($F = 2.84$; df = 3, 30, $P = 0.0545$). B) The statistical analysis is summarized in Table 3. * $P < 0.05$, ** $P < 0.01$.

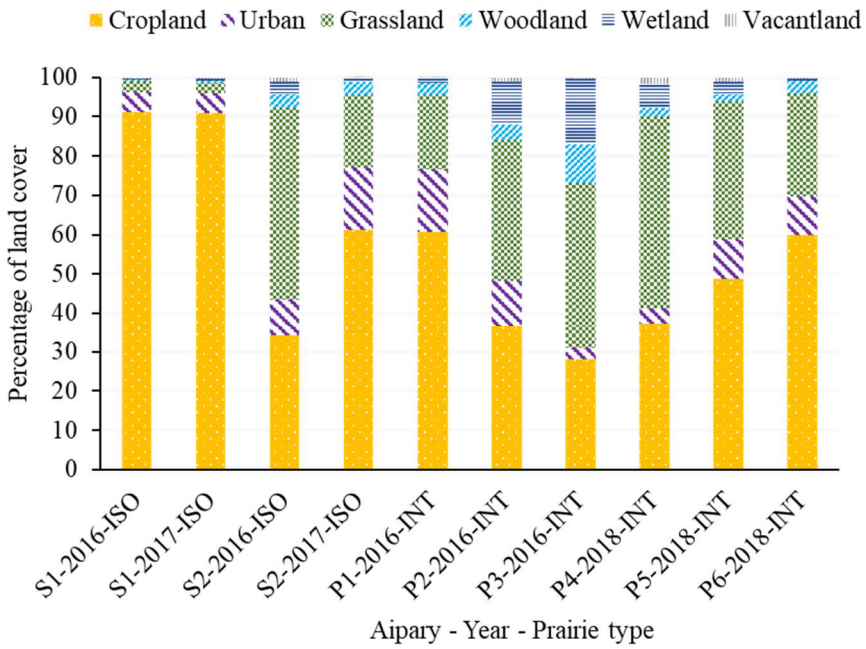

Figure 4. Percent of six general land cover type composing the landscapes around apiaries within a 1.6 km radius. ISO, isolated reconstructed tallgrass prairies, INT, integrated reconstructed tallgrass prairies.

Supplementary Tables and Figures

Supp. Table 1. Summary of prairie and apiary information.

Year	Prairie name	Prairie type [b]	County	Hectare [b]	Apiary symbol	Apiary latitude	Apiary longitude	Colonies per apiary	Pollen traps per apiary
2016	Meetz	ISO	Story	15	S1	42.059197	-93.541481	1	1
2016	Stargrass	ISO	Story	10.42	S2	41.999103	-93.554372	1	1
2016	Darnell-Holy Cross (CBG) [a]	INT	Polk	20.23	P1	41.791219	-93.401356	1	1
2016	Bailey-Carpenter (CBG)	INT	Polk	47.75	P2	41.759444	-93.373889	1	1
2016	Barrer (CBG)	INT	Polk	31.57	P3	41.731672	-93.352764	1	1
2017	Meetz [b]	ISO	Story	15	S1	42.056628	-93.541527	2	2
2017	Stargrass [b]	ISO	Story	10.42	S2	41.999103	-93.554372	2	2
2018	Engeldinger Marsh (CBG)	INT	Polk	36.42	P4	41.776394	-93.34951	4	2
2018	Kunze (CBG)	INT	Polk	69.20	P5	41.746154	-93.365855	4	2
2018	Lloyd Bailey (CBG)	INT	Polk	42.49	P6	41.731685	-93.368204	4	2

[a] CBG, Chichaqua Bottoms Greenbelt Conservation Area in Polk County in Iowa, USA. Two sets of three prairies in 2016 and 2018 in CBG were selected for installing our apiaries, respectively.

[b] We used the same prairies, i.e. Meetz and Stargrass, in both 2016 and 2017 for our apiary locations. ISO, isolated reconstruction tallgrass prairie. INT, integrated reconstruction tallgrass prairie.

Supp. Table 2. The land covers within 1.6 km radius grouped into six major types.

Cropland	Urban	Grassland	Woodland	Wetland	Vacant land
Corn, soybean, sweet corn, winter wheat, rye, oats, apple orchard	Developed/open space, developed/low intensity, developed/medium intensity, developed/high intensity	Alfalfa, non alfalfa hay, sod/grass seed, fallow, grass/pasture	Deciduous forest, evergreen forest, shrubland	Woody wetlands, herbaceous wetlands	Barren land, open water

Supp. Table 3. Pollen collection frequency in each month of the study across three years.

Month	2016	2017	2018
June	5 (3rd, 8th 16th, 23rd, **29th**) *	1 (**26th**)	2 (13th, **28th**)
July	4 (9th,15th, 23rd, **27th**)	2 (7th, **25th**)	2 (11th, **27th**)
August	3 (4th, 10th, **18th**)	2 (4th, **23rd**)	2 (11th, **28th**)
September	1 (**4th**)	3 (5th, 16th, **25th**)	1 (**7th**)

* Total number of collection (the exact collection date) within a month. The dates in bold were selected for determining the variation in diversity and abundance across months.

Supp. Table 4. Plant species included in our reference pollen library that were collected in Iowa during 2015-2018.

Family name	Scientific name	Common name	Plant type	Nativeness [a]	Invasiveness [b]
Amaranthaceae	*Amaranthus tuberculatus*	Common waterhemp	Herb	Native	Invasive
Asteraceae	*Ambrosia artemisiifolia*	Common ragweed	Herb	Native	Invasive
Asteraceae	*Ambrosia trifida*	Giant ragweed	Herb	Native	Invasive
Convolvulaceae	*Ipomoea nil*	Japanese morning glory	Herb	Native	Invasive
Brassicaceae	*Lepidium virginicum*	Virginia pepperweed	Herb	Native	Invasive
Cucurbitaceae	*Sicyos angulatus*	Bur cucumber	Herb	Native	Invasive
Asteraceae	*Achillea millefolium*	Yarrow	Herb	Native	Noninvasive
Asteraceae	*Ageratina altissima*	White snakeroot	Herb	Native	Noninvasive
Fabaceae	*Amorpha canescens*	Lead plant	Herb	Native	Noninvasive
Ranunculaceae	*Anemone canadensis*	canada anemone	Herb	Native	Noninvasive
Fabaceae	*Baptisia alba*	White wild indigo	Herb	Native	Noninvasive
Bignoniaceae	*Catalpa speciosa*	Northern catalpa	Tree or shrub	Native	Noninvasive
Fabaceae	*Chamaecrista fasciculata*	Partridge pea	Herb	Native	Noninvasive
Santalaceae	*Comandra umbellata*	Bastard toadflax	Herb	Native	Noninvasive
Asteraceae	*Coreopsis palmata*	Prairie coreopsis	Herb	Native	Noninvasive
Cucurbitaceae	*Cucurbita pepo*	Pumpkin	Herb	Native	Noninvasive
Fabaceae	*Dalea candida*	White prairie clover	Herb	Native	Noninvasive
Fabaceae	*Dalea purpurea*	Purple prairie clover	Herb	Native	Noninvasive
Fabaceae	*Desmanthus illinoensis*	Prairie minosa	Herb	Native	Noninvasive
Fabaceae	*Desmodium canadense*	Showy tick-trefoil	Herb	Native	Noninvasive
Rosaceae	*Drymocallis arguta*	Prairie cinquefoil	Herb	Native	Noninvasive
Asteraceae	*Echinacea pallida,*	Pale purple cone flower	Herb	Native	Noninvasive
Asteraceae	*Echinacea purpurea*	Purple coneflower	Herb	Native	Noninvasive
Asteraceae	*Erigeron strigosus*	Prairie fleabane	Herb	Native	Noninvasive
Apiaceae	*Eryngium yuccifolium*	Rattle snake master	Herb	Native	Noninvasive
Rosaceae	*Fragaria vesca*	Wild strawberry	Herb	Native	Noninvasive
Asteraceae	*Helianthus annuus*	Common sunflower	Herb	Native	Noninvasive
Asteraceae	*Helianthus decapetalus*	Thinleaf sunflower	Herb	Native	Noninvasive
Asteraceae	*Helianthus grosserratus*	Sawtooth sunflower	Herb	Native	Noninvasive

Supp. Table 4. Continued.

Family name	Scientific name	Common name	Plant type	Nativeness [a]	Invasiveness [b]
Asteraceae	*Heliopsis helianthoides*	False sunflower	Herb	Native	Noninvasive
Iridaceae	*Iris versicolor*	Blue flag	Herb	Native	Noninvasive
Fabaceae	*Lespedeza capitata*	Round-headed bush clover	Herb	Native	Noninvasive
Lamiaceae	*Monarda fistulosa*	Wild bergomot	Herb	Native	Noninvasive
Onagraceae	*Oenothera biennis*	Evening primrose	Herb	Native	Noninvasive
Asteraceae	*Penstemon digitalis*	Foxglove beard tongue	Herb	Native	Noninvasive
Polemoniaceae	*Phlox paniculata*	Garden phlox	Herb	Native	Noninvasive
Lamiaceae	*Pycnanthemum virginianum*	Virginia mountain mint	Herb	Native	Noninvasive
Asteraceae	*Ratibida pinnata*	Prairie coneflower	Herb	Native	Noninvasive
Rosaceae	*Rosa blanda*	Prairie rose	Tree or shrub	Native	Noninvasive
Asteraceae	*Rudbeckia hirta*	Black-eyed susan	Herb	Native	Noninvasive
Adoxaceae	*Sambucus spp.*	Elderberry	Tree or shrub	Native	Noninvasive
Asteraceae	*Silphium integrifolium*	Rosinweed	Herb	Native	Noninvasive
Asteraceae	*Silphium laciniatum*	Compass plant	Herb	Native	Noninvasive
Asteraceae	*Silphium perfoliatum*	Cup plant	Herb	Native	Noninvasive
Asteraceae	*Solidago canadensis*	Canada goldenrod	Herb	Native	Noninvasive
Asteraceae	*Solidago rigida*	Stiff goldenrod	Herb	Native	Noninvasive
Asteraceae	*Solidago speciosa*	Showy goldenrod	Herb	Native	Noninvasive
Asteraceae	*Symphyotrichum ericoides*	White heath aster	Herb	Native	Noninvasive
Malvaceae	*Tilia americana*	Basswood	Tree or shrub	Native	Noninvasive
Commelinaceae	*Tradescantia virginiana*	Spider wort	Herb	Native	Noninvasive
Lamiales	*Verbena stricta*	Hoary vervain	Herb	Native	Noninvasive
Asteraceae	*Vernonia noveboracensis*	Iron weed	Herb	Native	Noninvasive
Violaceae	*Viola papilionacea*	Wild violet	Herb	Native	Noninvasive
Apiaceae	*Zizia aurea*	Golden Alexander	Herb	Native	Noninvasive
Malvaceae	*Abutilon theophrasti*	Velvetweed	Herb	Nonnative	Invasive
Asteraceae	*Carduus nutans*	Musk thistle	Herb	Nonnative	Invasive

Supp. Table 4. Continued.

Family name	Scientific name	Common name	Plant type	Nativeness [a]	Invasiveness [b]
Amaranthaceae	*Chenopodium album*	Common lambsquarters/pigweed	Herb	Nonnative	Invasive
Asteraceae	*Cirsium arvense*	Canada thistle	Herb	Nonnative	Invasive
Asteraceae	*Cirsium vulgare*	Bull thistle	Herb	Nonnative	Invasive
Convolvulaceae	*Convolvulus arvensis*	Field bindweed	Herb	Nonnative	Invasive
Apiaceae	*Daucus carota*	Queen Anne's lace	Herb	Nonnative	Invasive
Fabaceae	*Melilotus albus*	White sweet clover	Herb	Nonnative	Invasive
Fabaceae	*Melilotus officinalis*	Yellow sweet clover	Herb	Nonnative	Invasive
Apiaceae	*Pastinaca sativa*	Wild parsnip	Herb	Nonnative	Invasive
Polygonaceae	*Persicaria maculosa*	Redshank	Herb	Nonnative	Invasive
Solanaceae	*Physalis peruviana*	Cape gooseberry	Herb	Nonnative	Invasive
Plantaginaceae	*Plantago lanceolata*	Ribwort plantain	Herb	Nonnative	Invasive
Caryophyllaceae	*Saponaria officinalis*	Bouncing bet	Herb	Nonnative	Invasive
Caryophyllaceae	*Silene latifolia*	White campion	Herb	Nonnative	Invasive
Asteraceae	*Sonchus arvensis*	Field sow thistle	Herb	Nonnative	Invasive
Asteraceae	*Taraxacum officinale*	Common dandelion	Herb	Nonnative	Invasive
Asteraceae	*Tragopogon dubius*	Yellow salsify	Herb	Nonnative	Invasive
Asparagaceae	*Asparagus officinalis*	Asparagus	Herb	Nonnative	Noninvasive
Brassicaceae	*Brassica napus*	Rapaseed	Herb	Nonnative	Noninvasive
Asteraceae	*Cichorium intybus*	Common chicory	Herb	Nonnative	Noninvasive
Fabaceae	*Glycine max*	Soybean	Herb	Nonnative	Noninvasive
Liliaceae	*Lilium lancifolium*	Tiger lily	Herb	Nonnative	Noninvasive
Fabaceae	*Medicago sativa*	Alfalfa	Herb	Nonnative	Noninvasive
Apiaceae	*Myrrhis odorata*	Sweet cicely	Herb	Nonnative	Noninvasive
Papaveraceae	*Papaver somniferum*	Opium poppy	Herb	Nonnative	Noninvasive
Fabaceae	*Phaseolus vulgaris*	Green bean	Herb	Nonnative	Noninvasive
Fabaceae	*Securigera varia*	Crown vetch	Herb	Nonnative	Noninvasive
Fabaceae	*Trifolium hybridum*	Alsike clover	Herb	Nonnative	Noninvasive
Fabaceae	*Trifolium incarnatum*	Crimson clover	Herb	Nonnative	Noninvasive

Supp. Table 4. Continued.

Family name	Scientific name	Common name	Plant type	Nativeness [a]	Invasiveness [b]
Fabaceae	*Trifolium pratense*	Red clover	Herb	Nonnative	Noninvasive
Fabaceae	*Trifolium repens*	White clover	Herb	Nonnative	Noninvasive
Scrophulariaceae	*Verbascum thapsus*	Common mullein	Herb	Nonnative	Noninvasive
Poaceae	*Zea may*	Corn	Herb	Nonnative	Noninvasive
Salicaceae	*Salix* spp.	Willow	Tree or shrub	Unknown	Noninvasive

[a] Nativeness and [b] invasiveness of each plant was decided based on the information in Plants Database provided by Natural Resources Conservation Service of United States Department of Agriculture https://plants.sc.egov.usda.gov/java/.

Supp. Table 5. Mean percent of pollen that was unidentified in our study.

Taxa	2016 (Mean ± SE, n = 5)				2017 (Mean ± SE, n = 2)				2018(Mean ± SE, n = 3)			
	Jun	Jul	Aug	Sept	Jun	Jul	Aug	Sep	Jun	Jul	Aug	Sep
UIPT1*	0.08 ± 0.05	0.02 ± 0.02	0.13 ± 0.13	0	0	45.7 ± 44.99	0.46 ± 0.46	0.67 ± 0.67	0.44 ± 0.41	0	1.17 ± 0.78	0
UIPT2	0	0	0.02 ± 0.01	0	0	0	0	0	0	0	0	0
UIPT3	0.01 ± 0.01	0	0	0	0	0	0	0	0	0	0	0
UIPT4	0.03 ± 0.03	0	0	0	0	0	0	0	0	0	0	0
UIPT5	11.09 ± 4.8	0.01 ± 0.01	0.08 ± 0.08	0	0	0	0	0	0	0	0	0
UIPT6	0.6 ± 0.59	0	0	0	0	0	0	0	0	0	0	0
UIPT7	7.82 ± 4.52	0	0	0	0	0	0	0	0	0	0	0
UIPT8	0.11 ± 0.07	0	0	0	0	0	0	0	0	0	0	0
UIPT9	0.01 ± 0.01	0	0	0	0	0	0	0	0	0	0	0
UIPT10	0	0.67 ± 0.67	0	0	0	0	0	0	0	0	0	0
UIPT11	0	0.01 ± 0.01	0.59 ± 0.59	2.75 ± 1.22	0	0	0	0	0	0	0	0
UIPT12	0	0.26 ± 0.25	0.03 ± 0.03	0	0	0	0	0	0	0	0	0
UIPT13	0.8 ± 0.58	0.04 ± 0.04	0	0	0	0	0	0	0	0	0	0
UIPT14	0	0	0	0.18 ± 0.18	0	0	0	0.02 ± 0.02	0	0	21.07 ± 8.4	0
UIPT15	0.24 ± 0.15	0	0.02 ± 0.02	0	0	0	0	0	0	0	0	0
UIPT16	1.82 ± 1.35	0	0	0	0	0	0	0	0	0	0	0
UIPT17	0	0	0.02 ± 0.02	0	0	0	0	0	0	0	0	0
UIPT18	0.3 ± 0.11	0	0	0	0	0.35 ± 0.35	0	0	4.01 ± 2.09	0.6 ± 0.32	0	0
UIPT19	0	0	0	0	0	0	0	0.03 ± 0.03	0	0	0	0
UIPT20	0	0	0	0	0	0	0	1.61 ± 1.61	0	0	0	0
UIPT21	0	0	0	0	0	0	0	0	0	0.05 ± 0.05	0	0
UIPT22	0	0	0	0	0	0	0	0	1.32 ± 1.32	0	0	0
UIPT23	0	0	0	0	0	0	0	0	0.13 ± 0.13	0.04 ± 0.04	0.19 ± 0.19	0
UIPT24	0	0	0	0	0	0	0	0	0.31 ± 0.18	0	0	0
UIPT25	0	0	0	0	0	0	0	0	0.66 ± 0.66	0	0	0
UIPT26	0	0	0	0	0	0	0	0	0.04 ± 0.04	0	0	0
UIPT27	0	0	0	0	0	0	0	0	0.65 ± 0.42	0	0	0

Supp. Table 5. Continued.

Taxa	2016 (Mean ± SE, n = 5)				2017 (Mean ± SE, n = 2)				2018(Mean ± SE, n = 3)			
	Jun	Jul	Aug	Sep	Jun	Jul	Aug	Sep	Jun	Jul	Aug	Sep
UIPT28	0	0	0	0	0	0	0	0	0	0	5.52 ± 1.89	1.12 ± 1.12
UIPT29	0	0	0	0	0	0	0	0	0	18.03 ± 16.6	0	23.55 ± 23.55
UIPT30	0	0	0	0	0	0	0	0	0	1.51 ± 1.51	0	0
UIPT31	0	0	0	0	0	0	0	0	0	0.26 ± 0.15	0	0
UIPT32	0	0	0	0	0	0	0	0	0	1.97 ± 1.94	3.21 ± 2.63	17.08 ± 4.14

* UIPT, unidentified pollen taxa.

Supp. Table 6. Comparison of pollen diversity among years using differences of least square means.

Effect	Year	Year	Estimate	Standard Error	DF	t Value	Pr > |t|	Adjustment method	Adjusted P value
Year	2016	2017	-0.75	0.9031	30	-0.83	0.4128	Tukey-Kramer	0.6873
Year	2016	2018	-2.0833	0.7374	30	-2.83	0.0083	Tukey-Kramer	0.022
Year	2017	2018	-1.3333	1.1659	30	-1.14	0.2618	Tukey-Kramer	0.4954

Supp. Table 7. Analysis of interaction of month and prairie type using differences of least square means.

Effect	Month	Prairie type	Prairie type	Estimate	Standard Error	DF	t value	P value
Month × Prairie type	June	INT [a]	ISO [b]	1.9899	0.8322	30	2.39	0.0233
Month × Prairie type	July	INT	ISO	0.1478	0.8322	30	0.18	0.8602
Month × Prairie type	August	INT	ISO	0.263	0.8322	30	0.32	0.7542
Month × Prairie type	September	INT	ISO	2.7081	0.8322	30	3.25	0.0028

[a] Integrated reconstruction tallgrass prairie

[b] Isolated reconstruction tallgrass prairie

CHAPTER 5. PRAIRIE STRIPS IMPROVE BIODIVERSITY, AND HONEY BEE FORAGE AND HEALTH IN AGRICULTURAL LANDSCAPES

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Authors' Contributions

GZ, CJM, AGD, LSM, ALT, MEO designed this study. GZ inspected apiaries and collected data on colony weight, bee population, Varroa mite population, queen losses and collected nurse bees for body lipid content analysis and pollen samples for estimating pollen diversity and abundance. GZ identified the taxonomic origin of pollen and measured the lipid content of nurse bees. GZ monitored the overwinter mortality. CJM surveyed flowering plants to estimate the floral diversity and abundance. GZ analyzed all the data and drafted manuscript.

Abstract

Beekeepers have experienced high annual losses and historical declines in the USA and Europe, in part due to reductions in floral resources used by honey bees. Increased agricultural production that removes non-cropped areas that contained flowering plants contributes to this

reduction. Integration of native perennial plants (e.g. prairie strips) into cropland is a conservation approach which reduces the impact of annual crop production on several environmental factors, including the movement of sediment and nutrients from the field into the watershed. Prairie strips also increase biodiversity, especially the diversity and abundance of wild, native pollinators. A multi-year, replicated, longitudinal study of apiaries placed in commercial corn or soybean fields with and without prairie strips was conducted to determine if prairie strips enhanced individual honey bee and colony health, an nonnative species in the USA. Multiple indicators of bee health were monitored throughout the season, including diversity and quantity of pollen collected by colonies, individual bee nutritional state (via nurse bee lipid content), and colony health (via weight and population growth). Colonies kept at a crop field with prairie strips had a greater average colony weight and larger worker-bee populations. Abundance of pollen may have contributed to this response, as colonies kept in prairie strips collected more pollen that included several plant species found in the prairie strips. These data suggest that prairie strips could be a solution to support conservation goals within farmland while simultaneously improving honey bee colony health.

Key words

Honey bees, prairies strips, nutrition, pollen, colony weight, population, overwinter survival

Introduction

Globally, the replacement of natural and semi-natural land with agricultural has been identified as a major factor in declining biodiversity worldwide (Foley et al. 2005, Lanz et al. 2018). At the same time, agricultural production heavily relies on ecosystem services supported by biodiversity, such as crop pollination by a community of insect pollinators (Power 2010), and this reliance is increasing as the growing worldwide human population requires more and more agricultural products (Harrison et al. 2014). To counter declining biodiversity and enhance

delivery of ecosystem services to agricultural land, initiatives that aim to reintegrate biodiversity into agriculture are receiving public and academic attention. Two prominent examples of conservation programs include the Conservation Reserve Program (CRP) in the USA and Agri-Environmental Scheme (AES) in European Union, both of which, since the 1980s, have encouraged farmers to convert erodible or unproductive farmland into conservation habitat by incentives of cost-share and rent payment (Dale et al. 2010, Whittingham 2011, Johnson et al. 2016). In general, these conservation programs can enhance ecosystem services and biodiversity on farmland through the integration of semi-natural or natural habitats (Boatman et al. 2008, Dale et al. 2010, Whittingham 2011, Ekroos et al. 2014, Batáry et al. 2015), though the response varies by taxa (Kleijn et al. 2006). Efforts to improve the ecosystem services delivered by insects through this integration have been proposed as a way to couple improvements to agriculture with conservation goals (Isaacs et al. 2009). Reintegration of natural habitat into agricultural landscapes has enhanced diversity, abundance or health of pollinators which can provide potential benefits to the pollination of crops or non-crop plant in a landscape (Haaland et al. 2011, Whittingham 2011, Scheper et al. 2014, Thogmartin et al. 2017, Otto et al. 2018, Ricigliano et al. 2019).

Managed honey bees are a key for the pollination of crops throughout the world while beekeepers suffer high annual colony mortality in US and Europe (Neumann and Carreck 2010, vanEngelsdorp and Meixner 2010, Calderone 2012). Honey bees suffer from several sources of stress that are challenging for beekeepers to manage, like Varroa mite infestations (Guzmán-Novoa et al. 2010, Seitz et al. 2015, Kulhanek et al. 2017) and pesticide exposure (Mullin et al. 2010). An additional source of stress is a deterioration of reliable, diverse forage in the agricultural landscape (Naug 2009, Potts et al. 2010, vanEngelsdorp and Meixner 2010, Goulson

et al. 2015). Previous studies in the USA suggest that an average of 26 hectare farmland (USDA 2020) converted into native grassland that incorporates native perennial forbs supports healthier honey bee colonies (Otto et al. 2018, Ricigliano et al. 2019). Despite the success of CRP, maintaining incentives for farmers to voluntarily engage in this practice has been challenging, and CRP land cover has shrunk as many farmers have converted this land back to crop production when commodity prices reached historic highs (Fargione et al. 2009, Wright and Wimberly 2013, Otto et al. 2016).

In the USA, a conservation approach involving the integration of small patches of native, perennial vegetation into cropland (i.e. prairie strips), has been shown to enhance biodiversity and increase delivery of ecosystem services (Schulte et al. 2017). By removing a small portion of land from crop production, (10% of a given field) prairie strips can increase water holding capability and reduce soil and nutrient losses from cropland. (Hernandez-Santana et al. 2013, Gutierrez-Lopez et al. 2014, Zhou et al. 2014, Schulte et al. 2017). The limited amount of land converted from crop land and the low cost of maintenance contribute to the addition of prairie strips as a cost effective conservation practice (Tyndall et al. 2013, Schulte et al. 2017). Prairie strips were added to CRP as part of the 2018 US farm bill (the main agricultural and food policy tool by US government) , providing additional incentives for farmers to utilize this practice (FSA 2018).

In addition to affecting soil and nutrient loss from farmland, prairie strips increase biodiversity within a farm, including wild, native pollinators (Schulte et al. 2017, Kordbacheh et al. 2018). Native plants found in prairies are attractive to native pollinators (Tuell et al. 2008), indicative of a co-evolved relationship. Honey bees were brought to North America by European settlers and are kept across the continent. Honey bees have been observed to use perennial

flowering plants sown in a mix as a source of forage in Europe (Campbell et al. 2017), and are attracted to prairies in the Midwest US (Carr-Markell et al. 2020). Several studies have explored the impact of flowering habitat on crop pollination and protection (Tooker et al. 2020), however there is limited data on the impact of such habitat has on honey bee health and productivity. Within central Iowa, honey bee colonies provided access to contiguous prairies in a conservation area stretching up to 16 kilometers (Chichaqua Bottoms Greenbelt, Polk County, Iowa) did not suffer from a late season dearth of forage after crops bloomed (Dolezal et al. 2019a). That study provided honey bee access to prairies only from August to October. Data are lacking on whether season-long access to native, perennial flowering forbs in the form of prairie strips of a moderate size (usually < 5 hectares) will improve honey bee forage and colony health. In this study, we provide the first field-scale, longitudinal assessment of the realized impact of prairie strips integrated into farmland on honey bee health.

Materials and methods

Site selection

Approximately 85 % of the state of Iowa's land cover is committed to agricultural production, primarily monocultures of corn and soybean (USDA-NASS 2019), making this part of the Midwestern US one of the largest and most extensively cultivated regions in the world (Fritz et al. 2015) and a major target for improved, sustainable agricultural practices. Commercial crop fields with prairie strips were identified through the collaborating farmers and landowners participating in the STRIPS (Science-Based Trials of Row Crops Integrated with Prairie Strips) project at Iowa State University. To date, 65 farms have reconstructed prairies with help from the STRIPS project (referred to as 'prairie strips' sites herein). For this study, we selected a subset of these farms located within central Iowa with prairie strips seeded at least three years prior to our study to ensure sufficient ground biomass had emerged (information

available in website of STRIPS project, https://www.nrem.iastate.edu/research/STRIPS/).The number of farms with prairie strips used within a year was two, four and five farms during 2017-2019, respectively. The crop grown adjacent to the prairie strips was either soybean or corn (Supp. Table 1). The location, number and configuration of the prairie strips varied by location (Supp. Table 2). In general, the number of patches (i.e. strips) varied from two to six and were established in the field or at field edge in 2014-2016 by cooperator farmers (Supp. Table 2). The hectares of cropland converted to strips were also decided by the farmers, ranging from 0.77-4.47 hectare (Supp. Table 2). The seed mix for establishing prairie strips was also decided by farmers and varied to some extent. Thus, we performed a plant survey in a prairie strip at each farm used in our study to describe the plant composition. Management of the prairie strips was limited during the period in which this experiment was conducted, for example, strips were not mowed from May to October during 2017-2019 when forbs were blooming (Supp. Table 2). All but one farmer (SME prairie strips) burned their prairie plants in fall or spring (Supp. Table 2) to enhance diversity of native plants by suppressing populations of nonnative plants (Tix and Charvat 2005). At each prairie strips site, one strip of prairie was randomly selected for an apiary, placed within three meters from an adjacent crop, either soybean or corn.

An equal number of commercial fields without prairie prairies were identified as control sites within Iowa during 2017-2019. An apiary location was selected at the field margin of a control site planted with either soybean or corn. Because most foraging activity of honey bees occurs within 1.6 km radius (Couvillon et al. 2014, Danner et al. 2014), we selected sites (both control and prairie strip sites) that were at least 3.2 km apart to reduce the probability that the foraging range would overlap between honey bees kept at a given site.

Land cover measurement

Variation in landscape composition surrounding apiaries was previously shown to affect honey bee foraging activity and colony health within central Iowa (Dolezal et al. 2019a), a region used for this experiment. Therefore, we measured landscape composition around potential apiary sites using ArcMap 10.5.1 (Redlands, CA), to select sites that did not vary significantly in landscape composition. This allowed us to minimize a source of variation that could potentially affect the foraging by honey bee kept at apiaries installed at control and prairie strips sites. Land use data layers from the National Agricultural Statistics Service of United State Department of Agriculture USDA (NASS-USDA) website were used, with 30 × 30 m resolution https://nassgeodata.gmu.edu/CropScape/. We categorized various landscape compositions into five general land covers, including cropland, urban, grassland, woodland, wetland.

Plant and flower survey

To determine the potential flowering vegetation at both control and prairie strips sites, we randomly selected a location within a strip of prairie to begin a 1×100 m transect for a plant survey, and at a control site, the same size transect was selected at random from a field margin or grass water way next to field margin. Within each transect, we counted the number of flowering plant (only forbs) species and open flowers in the transect once a month from June to August resulting in three plant surveys during 2018 (Supp. Table 3). In 2019, we conducted additional surveys in June and September resulting in five plant surveys during 2019 (Supp. Table 3).

Colony and apiary preparation

In early May 2017, colonies were constructed with a "package" of bees (wire mesh box containing approximately 0.9 kg of adult bees with one honey bee queen) supplemented with honey at the Iowa State University (ISU) apiary at the Horticulture Research Farm in Ames, Iowa. In early June of 2018 and 2019, honey bee colonies were created from nucleus colonies

composing of brood, adult bees, one honey bee queen and honey. Both packages and nucleus colonies were Italian honey bees (*Apis mellifera ligustica*) and purchased from local beekeepers in Iowa.

We started each colony for these experiments within a single, ten-frame Langstroth hive box (depth, interior length and interior width being 24, 47 and 37cm, individually) to house the starting populations. Each colony had approximately 7,000 adult bees and a total colony weight (containing adult bees, wax, honey and/or brood) that varied by year; 6.5-12.0 kg, 5.8-9.7 kg and 7.7-13.2 kg in 2017, 2018 and 2019, respectively. To control for variation in the starting colony weight, individual colonies were assigned to an apiary resulting in a similar average colony weight among all apiaries (2017: control 8.56 ± 0 kg, prairie strips 7.88 ± 1.36 kg; 2018: control: 7.74 ± 0.07 kg, prairie strips 7.65 ± 0.05 kg; 2019: control 10.48 ± 0.03 kg, prairie strips 10.49 ± 0.07 kg). The number of colonies within an apiary varied by year; two in 2017 and four in 2018 and 2019. As the population and resources brought to the hive increased (nectar, pollen), additional hive boxes were added.

Once the colonies had been assigned to an apiary, the apiaries were randomly assigned to a prairie strip or field edge of a control site without prairie strips. The colonies comprising an apiary were kept on a wooden pallet and placed either within a prairie strip or on a field edge of a control farm within three days of being created at the ISU apiary. To ensure that the colonies at a site were independent experimental units, the distance between any apiaries always exceeded 3.2 km (a distance that minimizes foraging range overlap) (Couvillon et al. 2014).

Apiary monitoring and management

We monitored the growth of the apiaries by inspecting each colony one to three times per month with at least a week interval between each inspection from May to October in 2017-2019 (refer to Supp. Table 3 for details of inspection date). During each inspection, we collected the

following data: colony weight, immature and mature bee population, queen presence and *Varroa desctructor* (Varroa) population. A measurement of colony weight includes the combined weight of immature (including eggs, larvae and pupae) and mature bees, comb, pollen and honey. The majority of a honey bee colony's weight is attributed to the stores of honey during the summer and fall (McLellan 1977). We measured colony weight by subtracting the weight of all the hives' wood components (including box, frame, bottom board and lid) from total hive weight measured on a digital scale. New hive boxes added to help colonies with storage of incoming nectar were weighed before being included during the field season.

Bee population within a colony was estimated as another indicator of colony growth. We estimated two of the developmental stages of honey bees that can be found within a hive box; pupae and adults. The pupal stage is the last immature stage of a developing honey bee that reside within in wax cells of the frames kept within a hive box. We estimated immature bee population by measuring the number of pupa within cells capped by a thin layer of wax. During each inspection, a 43.2 cm × 20.1 cm piece of plexiglass with a gridded pattern was placed on top of each side of the frames to estimate the amount of capped cells by square centimeter.

We also estimated adult bee populations on each side of a frame, with each 'frame-side' being a unit of measurement. A 'frame-side' of bees was defined as one side of a frame being fully covered with adult honey bees. During the inspection of each frame for a given colony, we noted the presence of a paint-marked, adult queen. If the queen was not found, we also noted her presence based on the occurrence of eggs or young larvae of < 3 day old. We reported whether the colony was "queenless" (queen absent, no eggs or young larvae) or "queenright" (queen or eggs, young larvae present). If a colony was considered queenless during an inspection, a new queen was added to the colony within three days. We also collected approximately 300 young (1-

3 d old) adult bees from a frame with emerging adult bees from each colony. These bees were washed in 75 % alcohol to dislodge Varroa mites from the bees, in a container with a fine mesh allowing mites to pass through to the bottom of the cup, and the number of Varroa mites was counted. In addition to data collected at a site, we collected samples of 30-50 nurse bee to measure their lipid content with a lab-based assay. We collected the identified nurse bees based on their occurrence on a frame with open brood (larvae) in each colony. These samples were placed in a 15 ml plastic falcon tube on ice and after returning to the lab, they were stored at - 80 °C for body lipid content analysis (detailed below).

We did not provide any supplemental food to colonies from May to early October in 2017-2019, once colonies were moved from ISU apiaries to prairie strips or control sites. To prevent a potential breakout of Varroa mite in fall (Coffey et al. 2010, DeGrandi-Hoffman et al. 2016), colonies were treated with miticide, Apilife Var with thymol as active ingredient (Chemicals Laif SPA, Vigonza, Italy) twice in September during 2017. Colonies were treated with Apiguard (Vita Europe Ltd, Valdosta, USA) also with thymol as active ingredient once in late August and once in late September, respectively, during 2018 and 2019. The second miticide application was at least one week after the first application.

Measuring lipid content of nurse bees

Nurse bees, which are adult hives bees < 21 day old that specialize in brood feeding, eat a large portion of pollen stores in honey bee colonies and store abundant lipids in their fat bodies, allowing them to rear new brood (Toth and Robinson 2005). Total lipid content can be used as indicator of honey bee health (Dolezal et al. 2019a). To estimate lipid content, five bees from the sample of nurse bees collected from open brood (larvae) of each colony were crushed with liquid and was measured following the method used by (Toth and Robinson) with some slight modifications. Lipids of nurse bees were extracted with 5ml 2:1 chloroform:methanol in glass

vials for 24 h and residual bee tissue was filtered out by glass wool. The liquid lipid extract was adjusted to 6 ml by adding additional 2:1 chloroform:methanol. A volume of 0.1 ml lipid extract was reacted with 0.2 ml sulfuric in glass tubes in boiling water for 10 min, and then with 2 ml sulfophosphovanillin reagent in a dark laboratory location (lab bench drawer), at room temperature for 10 minutes. The absorbance of 0.2 ml reaction products was measured at 525 nm with a Synergy HT spectrophotometer (BioTek, Winooski, USA).

Collection and Identification of plant taxa from bee-collected pollen

Pollen traps (Brushy Mountain Bee Supply, Wilsonville, USA) were attached to the entrances of colonies to determine the amount and type of plant used as a source of pollen. When activated, traps remove pollen from individual honey bees as they re-enter the colony from a foraging trip. Traps were activated for a 24 h period, otherwise they were left inactivated and did not interfere with bees leaving or entering the colony. Traps were placed on both of the two colonies comprising an apiary during 2017 and two of the four colonies comprising an apiary in 2018 and 2019, with a colony selected at randomly to receive a trap. To limit the amount of disturbance on the normal foraging activities of honey bees, pollen traps were not activated during the day of an apiary inspection. One to three pollen samples were collected from each pollen trap every month from June to October in 2017-2019 (Supp. Table 3). Each sample was collected within 24 h without rain at an interval of at least a week. Each sample collected from a pollen trap after 24 h was weighed and stored at - 20 °C for subsequent identification of the plant source.

The diversity of plants represented within bee-collected pollen was determined based on morphological differences of the pollen observed under compound microscope. Pollen from each colony at an apiary collected on same day was pooled after they were weighed. A subsample of 2 g was removed from the pooled pollen for estimating plant diversity. The pollen

pellets of the 2 g subsample were sorted based on their color. Pellets of the same color were weighed and mounted to microscope slide using Calberla's fluid with fuchsin stain as the mounting reagent. Pollen mounted to glass slide was compared to pollen collected from the flowers collected from both study sites for identification. If the features of pollen collected by honey bees and flower pollen matched, the pollen of the same color was assigned with a plant taxa name. Corbicular pollen that could not be identified was given a unique identifier number, which was used for estimating plant diversity for each pollen source.

Experimental design and statistical analysis

We compared the percent of land covers in surrounding landscapes around apiaries kept at prairie strips with those at controls using *t*-test (JMP pro 14, SAS institute, Cary, USA). Each site during 2017-2019 was a replication. To study the effect of prairie strips on floral resource, we compared the total plant taxa and flower counts at prairie strips sites surveyed across seasons with those at controls using *t*-test (JMP pro 14, SAS institute, Cary NC). Each transect used for plant survey at each site in either 2018 or 2019 was a replication.

To study the effect of prairie strips on honey bee health, we treated each apiary during 2017-2019 as the unit of replication. Data for pollen diversity and quantity, colony weight were collected for all the years of study during 2017-2019. Data for bee population, Varroa mite and lipid content in nurse bees were collected during two years, i.e. 2018-2019. Because we inspected apiaries and collected samples on different dates in a month among years, we binned those different dates in a month into three sampling periods, i.e. early, middle and late, to facilitate the analysis of effects of different years and dates. Average values across multiple colonies within each apiary for pollen quantity, colony weight, bee population, Varroa mite population, lipid content in nurse bees were used for statistical analysis. Because the pollen diversity was measured on pooled pollen samples from two pollen traps, it was used for

statistical analysis without calculating the average across colonies within an apiary. We used repeated measures linear mixed-effect model with Proc Mixed in SAS (SAS institute, Cary, USA) to analyze the difference in pollen diversity and abundance, colony weight, bee population, Varroa mite levels, lipid content in nurse bees between controls and prairie strips. Pollen diversity and quantity, colony weight, bee population, Varroa mite population, lipid content in nurse bees were included in the model as response variables. The values of response variables were log transformed with base 10, square root transformed or non-transformed to make the data conform to normal distribution. For analyzing the data of pollen diversity and colony weight, main effects that contained prairie strips, year, sampling period and crops were included in the model. Because unequal number of sampling periods among three years of study during 2017-2019, a three way interaction (prairie strips × year × sampling period) cannot be corrected estimated, instead two-way interactions (prairie strips × year and prairie strips × sampling period) were used in the model. When analyzing data of pollen abundance, we selected an optimum model by excluding the effects that did not reach to $P = 0.05$ significant level, resulting in a model that included main effects that reached to a significance level and the interactions of these main effects. To analyze the effects of prairie strips on immature and mature bee population and lipid content in nurse bees, main effects such as prairie strips, year, sampling period and crops and a three-way interaction (prairie strips × year × sampling period) were included in the model. To analyze the variance of Varroa mite levels between control and prairie strips sites, the main effects such as prairie strips, year, and sampling period were all included in the model, as well as a three-way interaction of these three factors. The results from analysis of variance (ANOVA) informed the overall effects of prairie strips on those response variables. We used the post hoc *t*-test with the difference of least square means to estimate the effects of prairie

strips on pollen diversity and quantity, colony weight, bee population, Varroa mite population, lipid content in nurse in individual sampling period. We compared the seasonal apiary queen losses between control and prairie strips using *t* test upon the data collected from 2018 and 2019 (JMP pro 14, SAS institute, Cary NC). The seasonal apiary queen losses was calculated as total queen losses of an apiary composed of four colonies across the growing seasons.

Results

Land covers within the surrounding landscapes

We did not observe a significant difference in major land covers in a 1.6 km radius surrounding apiaries within the two treatment sites (i.e. prairie strips versus control) ($P > 0.05$, Supp. Table 4, Fig. 1, Supp. Fig. 1). Overall, cropland was the dominant land cover (around 78% in average).

Diversity and abundance of floral resource

A total of 13 flowering plant taxa were observed at control sites and 36 in prairie strips in 2018 and 2019 (Table 1). The number of flowering plant taxa found at prairie strips was significantly more diverse than those at control sites ($t = 6.08$, df =12.11; $P < 0.001$) (Fig 2a), as was the abundance of flowers ($t = 2.18$, df = 12.66; $P = 0.049$) (Fig 2b). Both number of plant taxa and abundance of flowers at prairie strips sites were five times higher than control sites.

Diversity and abundance of bee-collected pollen

From samples collected from 2017-2019, a total of 53 and 58 plant taxa represented in pollen collected by honey bee colonies at control and prairie strips sites, respectively, with 41 plant taxa shared between the two treatment sites (Fig. 3, Supp. Table 5). When samples were pooled for the entire growing season, the richness of plant taxa found in pollen collected by colonies did not differ significantly between control and prairie strip sites ($F = 0.75$, df = 1, 37.7; $P = 0.3921$) (Supp. Table 6, Fig. 4A). In each sampling period, the richness of plant taxa found

in pollen was not significantly different between control and prairie strips sites (post hoc t test $P > 0.05$, Supp. Table 8). Pollen diversity did not differ among years ($F = 2.56$, df = 2, 38.2; $P = 0.0901$) (Supp. Table 7) or by the crop (soybean versus corn) immediately adjacent to the apiaries ($F = 0.16$, df = 1, 37.6; $P = 0.6932$) (Supp. Table 6). Pollen diversity varied among sampling periods ($F = 12.93$, df = 7, 125; $P < 0.0001$) with a late season decline observed in September (Supp. Table 9).

Overall, colonies kept in prairie strips collected significantly more pollen than those at control sites during 2017-2019 ($F = 6.18$, df = 1, 50.3; $P = 0.0163$) (Supp. Table 6, Fig. 4B). Colonies kept in prairie strips collected significantly more pollen than those at control sites at a single sampling time period, i.e. middle August ($P = 0.0014$) (Supp. Table 8, Fig. 4B). Pollen abundance did not differ between years ($F = 0.56$, df = 2, 37.9; $P = 0.5784$) or between crop types (corn versus soybean) in which colonies were immediately adjacent ($F = 2.9$, df = 1, 39.2; $P = 0.0964$) (Supp. Table 7).

Plant taxa in prairie strips found in pollen collected by honey bees

Thirty six (36) taxa of flowering plants were observed in prairie strips and 18 were found in pollen collected by colonies kept in both prairie strip sites and control sites (Table 1). The only plant found in the pollen collected by honey bees and not present in the prairie strips was *Phlox pilosa*. Of those 18 plants, 14 taxa were not found in the surveys conducted at control sites. The remaining four taxa (*Dalea purpurea*, *Pastinaca sativa*, *Trifolium pretense*, *Trifolium repens*) were present in both prairie strips and control sites. Although only marginally significant, colonies kept in prairie strips collected more pollen from the 18 plants found prairie strips ($t = 2.08$, df = 12.17; $P = 0.058$) (Supp. Fig. 2) as well as from the subset of 14 plants unique to prairie strips ($t = 1.95$, df = 12.17; $P = 0.0746$) (Supp. Fig. 2).

Colony growth

The immature bee population was not significantly different between control and prairie strips for the entire season, during 2018-2019 ($F = 1.72$, df = 1, 7.83; $P = 0.2268$) (Supp. Table 6, Fig. 5A). Only in a single sampling period (middle July) was the immature bee population significantly larger at prairie strips compared to control sites ($t = 2.97$, df = 15.98; $P = 0.0091$) (Supp. Table 10). Immature populations did not differ by year ($F = 3.36$, df = 1, 26.4; $P = 0.078$) or by the crop adjacent to the apiaries ($F = 0.22$, df = 1, 27.7; $P = 0.6414$) (Supp. Table 6). Immature bee populations varied during the growing season with a sharp decrease in late season (Fig. 5A).

Overall, mature (adult) bee populations at prairie strips sites were larger than those at control sites ($F = 6.43$, df = 1, 19.3; $P = 0.0199$) during 2018-2019 (Supp. Table 6, Fig. 5B). Adult bee populations were larger in prairie strips than control sites during three specific sampling periods (early and middle July, and early September) ($P < 0.05$, Supp. Table 10). Adult bee populations increased during the growing season with the largest population observed in early October (Fig. 5B). Adult bee populations did not differ by year ($F = 0.61$, df = 1, 19.5; $P = 0.4457$) or by the crop adjacent to the apiaries $F = 0.54$, df = 1, 20; $P = 0.4702$ (Supp. Table 6).

Overall, colonies kept in prairie strips were significantly heavier than those at control sites ($F = 4.54$, df = 1, 24.7; $P = 0.0432$) during 2017-2019 (Supp. Table 6, Fig. 5C). In five sampling periods from late July to early September, colonies kept in prairie strips were significantly heavier than those at control sites ($P < 0.05$, Supp. Table 10, Fig. 5C). Colony weight did not differ by adjacent crop, corn versus soybean ($F = 0.12$, df = 1, 24.4; $P = 0.731$), but did vary by year ($F = 21.38$, df = 2, 25.3; $P < 0.0001$) (Supp. Table 6). Colony weight was significantly heavier in 2017 and 2019 than 2018, with no significant difference between 2017 and 2019 (multiple comparison with Tukey-Kramer adjustment, Supp. Table 11).

Nurse bee lipid content

Overall, the amount of total body lipids in nurse bees did not differ significantly between control and prairie strips site during 2018-2019 ($F = 1.05$, df = 1, 22.2; $P = 0.3167$) (Table 6, Supp. Fig. 3). At no sampling period did lipid concentration of nurse bees vary significantly between controls and prairie strips ($P > 0.05$, Supp. Table 10). Lipids in nurse bees differed by year with lipid content in 2019 being higher than that in 2018 ($t = -9.98$, df = 21.1; $P < 0.0001$). Lipids in nurse bees varied by sampling date ($F = 98.68$, df = 2, 28.1; $P < 0.0001$; multiple comparisons with Tukey-Kramer adjustment) (Supp. Table 12), with the greatest lipid content observed during early October.

Varroa mite infestation and queen losses

In general, Varroa mite populations were not significantly different between control and prairie strips sites throughout the season during 2018-2019 ($F = 0.34$; df = 1, 15.6; $P = 0.5657$) (Supp. Table 6, Supp. Fig. 4). Mite populations did not significantly differ between controls and prairie strips at any time point ($P > 0.05$) (Supp. Table 10). Mite populations in 2018 were larger than populations in 2019 ($t = 4.08$; df = 15.6; $P = 0.0009$). Mite populations varied significantly by time (month), with the highest populations observed in August ($F = 18.41$, df = 2, 27; $P < 0.0001$; multiple comparison with Tukey-Kramer adjustment) (Supp. Table 13). Queen losses across seasons during 2018-2019 was not significantly different between controls and prairie strips ($t = 0$, df = 15.56; $P = 1$) (Supp. Fig. 5).

Discussion

Lack of quantity or diversity of forage in an agricultural landscape is a challenge to honey bee health, and one way to address this issue is to create habitat with diverse flowering plants. This study explored if a conservation practice, i.e. integrating prairie strips into cropland in an effort to reduce soil and fertilizer loss, would increase floral biodiversity and if this

biodiversity would benefit honey bees. Our results suggest that the increased floral diversity and subsequent abundance enhanced forage availability and colony growth.

Potential confounding factors did not interfere with main findings

We attempted to control or limit variation from other factors that may confound the estimate of honey bee colony growth including the landscapes compositions around a site, Varroa mite infestations and queen losses. Honey bees can find forage in both the reconstructed prairies but also the surrounding landscapes. The absence of a difference in landscape composition surrounding apiaries at strips and control sites suggests floral resources in the surrounding landscapes were unlikely to contribute to differences observed among the various metrics of colony growth. On the contrary, diverse and abundant floral resources at strips sites were more likely the contributor to enhanced forage at prairie strips. Varroa mites are considered to be the most destructive pest for beekeeping in the USA (Kulhanek et al. 2017), but were not observed in significant amounts in out apiaries and did not vary significantly between control and prairie strips. The failure of honey bee queens to survive a growing season are included as a response from the various stressors that affect colony mortality, such as pathogens and pesticide exposure (DeGrandi-Hoffman et al. 2013, Williams et al. 2015, Esmaeil et al. 2017). The lack of a difference in queen losses between controls and prairie strips suggests this also did not significantly contribute to the difference in forage and colony growth between sites.

Prairie strips enhanced both diversity and abundance of floral resources

Plant and floral survey indicated that prairie strips were well established at each site and harbored more diverse and abundant floral resources than control sites. The extant to which this increase in native plant abundance alone was responsible for difference in colony performance requires an exploration of which plants were used by honey bees as forage. By comparing plants represented in the bee-collected pollen with plants observed to be present in prairie strips, we

estimated which plant taxa were used by honey bees. Our results suggest that honey bees in prairie strips used only half of the floral taxa in prairie strips for pollen (18 out 36 plant taxa). Also, apiaries kept at controls sites used plants found in prairie strips (18 plant taxa). This observation suggests that either those plants are not limited to prairie strips (e.g. also found in roadsides or other forage habitats in the surrounding landscapes), or honey bees from apiaries kept at a control site are able to fly to neighboring prairie strips and use them for forage. Although we attempted to select control sites that were sufficiently far from strips sites to be independent, the value of the forage within the prairie strips may support foraging activity of bees from control sites across such a great distance (Dolezal et al. 2019a). There are other reconstructed native habitats across Iowa that may contain those flowering plants like the CRP-42 program (USDA-FSA 2020) and roadside enhanced with floral resources by the Iowa Department of Transportation. However, the land cover data layer provided by Cropscape (USDA-NASS) did not allow us to identify CRP programs among the types of land use reported or capture the area of roadside with enhanced plant diversity because of the low resolution of the satellite imagery (30 m × 30 m, usually larger than the width of a roadside).

Prairie strips enhanced forage abundance for colonies

Honey bees kept in prairie strips collected more pollen than those at control sites, despite colonies at both locations using plants found in the prairie strips. Honey bees have a foraging range that can extend several kilometers away from their hive, so it may be possible that honey bees kept at control sites compensated for the immediate lack of quantity and diversity of flowering plants by extending their foraging range. The lower amount of pollen collected by honey bees at control sites suggests the limitations of this strategy for improving pollen availability. The failure of using a low level of floral resources to collect abundant pollen at the control sites hinted at the importance of quantity and diversity of plant for maintaining a healthy

colony. As the only source of macronutrients including proteins, lipids and micronutrients, pollen may play a key role in maintaining colony health, especially in light of stressors commonly experienced in agricultural landscape of Iowa, such as pesticide (Schmehl et al. 2014). To what extent these colonies were using diverse plants for pollen to help enhance immunity to pathogens commonly found during beekeeping in this region is not clear. However, previous studies demonstrated that a diverse pollen diet can increase honey bees' resistance to pathogens (Di Pasquale et al. 2013, Dolezal et al. 2019b, Zhang et al. 2020).

Prairie strips enhanced colony growth

Colonies in prairie strips had larger adult bee populations than those at control sites, suggesting that prairie strips enhance colony population size. Interestingly, the immature bee populations were similar between control and prairie strips, suggesting that an improvement in reproduction is not responsible for the difference in adult bee populations. Differences in adult worker survival or lifespan could explain these results. More active honey bees have a shorter lifespan (Schmid-Hempel and Wolf 1988). Although we did not measure individual adult survival, difference in mortality rates may occur if adult bees at control sites had to forage within a larger area to collect sufficient resources to sustain colony growth, resulting in depleting their reserve energy leading to a mortality. Our data suggest that the differences in adult population may be a function of the more immediate sources of forage provided by prairie strips to the colonies placed adjacent to them.

Elucidating the mechanism by which larger bee populations were produced at the prairie strips could lead to practices to promote more sustainable beekeeping. Larger populations can contribute to a higher probability of overwintering survival (Döke et al. 2019). There may be a financial gain for beekeepers to place apiaries at prairie strips, as larger populations improve the rates at which they can charge for renting their hives for crop pollination (Goodrich 2019).

Regardless of where the apiaries were located, all colonies gained weight from June through August. Although we did not harvest honey from these colonies, the timing of this weight change was consistent with honey being the greatest contributor to this change especially during summer months as observed in our study (Supp. Table 13) and previous studies (McLellan 1977). It was beyond the scope of this study to determine what plants contributed to the nectar that ultimately produced this weight change. Honey bee colonies located next to soybean fields in central Iowa gained weight during this same period (Dolezal et al. 2019a) in landscapes with a percentage of cropland (84%) similar to the landscapes surrounding the sites used in this study. As noted by Dolezal et al. (2019a), plants that are in bloom during this time within central Iowa were soybean and clover, two common sources of nectar for honey bees. Colonies reached a greater peak weight when placed within prairie strips than controls. This difference may be due to the proximity of the prairies strips than the more grass-dominated plant community of controls. This was remarkable as the prairie strips are of a modest size (average 2 hectares, Supp. Table 2), and are comprised of multiple plant species that make up a fraction of the total plant community across Iowa. Our pollen data suggest that honey bees at prairie strips sites used plants both in apiaries and surrounding landscapes. To what extant the forage in the prairie strip supplemented the nectar typically used by honey bees in central Iowa is unclear. It is possible that colonies at prairie strips had more nectar-producing plants closer to them, allowing them to more efficiently accumulate honey stores. Since we cannot confirm the plant source of the nectar, we cannot test this hypothesis. Other mechanisms may help explain the capacity of colonies at prairie strips to produce more honey. For example, foraging workers may be healthier due to a more abundant pollen diet that improved their response to multiple stressors, resulting in more efficient workers.

Although, colonies at control sites generally collected less pollen than those at prairie strips, the lipid content of nurse bees at control sites and prairie strips was similar, suggesting that nurse bees may have met their nutritional needs at all sites. Pollen availability in hives can affect egg-laying by queen bees, which are fed by nurse bees (Fine et al. 2018). Larvae are also fed by nurse bees, thus the amount of capped brood present can indicate levels of nurse bee feeding activity. The lack of significance difference in capped brood (pupae) between controls and prairie strips (at all but except for one sampling period) also suggests that nurse bees at control sites had the nutritional capability to rear larvae and queens at similar levels at control and strips sites. It is possible that nurse bees may benefit in other ways from access to the more abundant forage at prairie strips, such as improved immunity to pathogens; this idea awaits further research.

This was the first study to demonstrate that cultivation of a native plant community can benefit the nonnative honey bee. Prairie strips were established at these locations with collaboration from the STRIPS project (https://www.nrem.iastate.edu/research/ STRIPS/), which helped the landowner select seed comprised of flowering forbs and grasses native to the North Central US. Three years after strips were established, the plant community has more flowering perennial plants than is typically found in the Iowa landscape (information available through the link for STRIP project noted above), which can produce a more abundant wild pollinator community (Schulte et al. 2017). Although honey bee health is an important goal for agriculture and apiculture, it is important to assess how adding these nonnative pollinators to a tract of native habitat will affect wild pollinators. It has been suggested that honey bees may compete with other pollinators for forage (Geldmann and González-Varo 2018). Empirical studies have shown mixed results; the presence of honey bees has been observed to have no effect or a

negative effect on wild pollinators (Mallinger et al. 2017). In Iowa, no effect of honey bee presence was observed on the community of wild bees found in soybean fields and vegetable farms (St Clair et al. 2020), or in prairies (Pritchard et al. in prep). As the effect of honey bees on wild pollinator can be context-dependent (Mallinger et al. 2017), it is important to investigate if prairie strips can be used for both supporting both honey bees and wild pollinators. Only half of the flowering plants found in prairies were used as a source of pollen (though we lack information on nectar), suggesting that honey bees may not deplete the pollen resources needed for wild pollinators. Future studies should determine the extent to which honey bees use the flowering plants available within prairies, such that competition is constrained to a subset of plants within them.

In conclusion, colonies kept at prairie strips collected more forage and grew to a larger size compared to colonies kept in conventional row crop fields (controls). This suggests prairie strips can significantly enhance honey bee health and bring more financial benefits to beekeepers through a possible higher honey yield and larger bee populations. Commercial beekeepers usually maintain apiaries of a larger size than those represented in this study (i.e. more than four colonies per apiary). If farms with prairie strips are to be recommended for commercial-scale beekeeping, future studies should investigate the carrying capacity of these sites for supporting larger apiaries. If confirmed, prairie strips have the potential to become an important approach for conserving biodiversity and honey bee health in the Midwestern USA where landscapes are dominated by monoculture row crop production.

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Tables and Figures

Table 1. Flowering plants found at control and prairie strips sites and if these plants found in bee-collected pollen.

Plant taxa Scientific name (common name)	Plant found in floral survey		Plant found in bee-collected pollen	
	Control	Strips	Control	Strips
Aesclepias syriaca (common milkweed)	Y[a]	N	N	N
Aquilegia spp. (columbine)	N	Y	N	N
Asclepias incarnata (swamp milkweed)	N	Y	N	N
Asclepias tuberosa (butterfly milkweed)	N	Y	N	N
Chamaecrista fasciculata (partridge pea)	N	Y	Y	Y
Coreopsis tripteris (tall coreopsis)	N	Y	N	N
Dalea purpurea (purple prairie clover)	Y	Y	Y	Y
Desmanthus illinoensis (Illinois bundleflower)	N	Y	N	N
Desmodium canadense (showy tick trefoil)	N	Y	N	N
Echinacea pallida (pale purple coneflower)	N	Y	Y	Y
Erigeron annuus (eastern daisy fleabane)	N	Y	N	N
Eryngium yuccifolium (rattlesnake master)	N	Y	N	N
Helianthus grosseserratus (sawtooth sunflower)	N	Y	Y	Y
Heliopsis helianthoides (ox-eye)	N	Y	Y	Y
Liatris pycnostachya (prairie blazing star)	N	Y	N	N
Monarda fistulosa (wild bergamot)	N	Y	Y	Y
Phlox pilosa (prairie phlox)	Y	N	Y	Y
Pycnanthemum virginianum (virginia mountain mint)	N	Y	Y	Y
Ratibida pinnata (yellow coneflower)	N	Y	N	N
Rosa multiflora (multiflora rose)	Y	N	N	N
Rudbeckia hirta (black-eyed Susan)	N	Y	N	N
Rudbeckia triloba (brown eyed susan)	N	Y	N	N

Table 1. Continued.

Plant taxa Scientific name (common name)	Plant found in floral survey		Plant found in bee-collected pollen	
	Control	Strips	Control	Strips
Silphium laciniatum (compass plant)	N	Y	Y	Y
Silphium perfoliatum (cup plant)	N	Y	Y	Y
Siphium integrifolium (rosinweed)	N	Y	Y	Y
Solidago spp. (goldenrod)	N	Y	Y	Y
Symphyotrichum ericoides (white heath aster)	N	Y	N	N
Symphyotrichum laeve (smooth blue aster)	N	Y	N	N
Tradescantia spp. (spiderwort)	N	Y	N	N
Verbena stricta (hoary vervain)	N	Y	N	N
Zizia aurea (golden alexanders)	N	Y	N	N
Cichorium intybus (chickory)	Y	N	N	N
Cirsium spp (thistle)	N	Y	Y	Y
Convolulus spp (morning glory)	Y	Y	N	N
Daucus carota (Queen Anne's lace)	Y	N	N	N
Lactuca serriola (prickly lettuce)	Y	N	N	N
Lotus corniculatus (birdsfoot trefoil)	N	Y	Y	Y
Medicago lupulina (black medic)	Y	N	N	N
Medicago sativa (alfalfa)	Y	N	N	N
Melilotus officinalis (yellow sweetclover)	N	Y	Y	Y
Pastinaca sativa (wild parsnip)	Y	Y	Y	Y
Taraxacum officinale (dandelion)	N	Y	Y	Y
Trifolium pratense (red clover)	Y	Y	Y	Y
Trifolium repens (white clover)	Y	Y	Y	Y
Total taxa found	13	36	19	19

[a] Y and N under column "Plant found in floral survey" indicated that plant was present (Y) or absent (N) at sites of this duty, e.g. control versus prairie strips. Y and N under column "Plant

found in bee-collected pollen" indicated that plant was found in pollen collected by honey bees at sites.

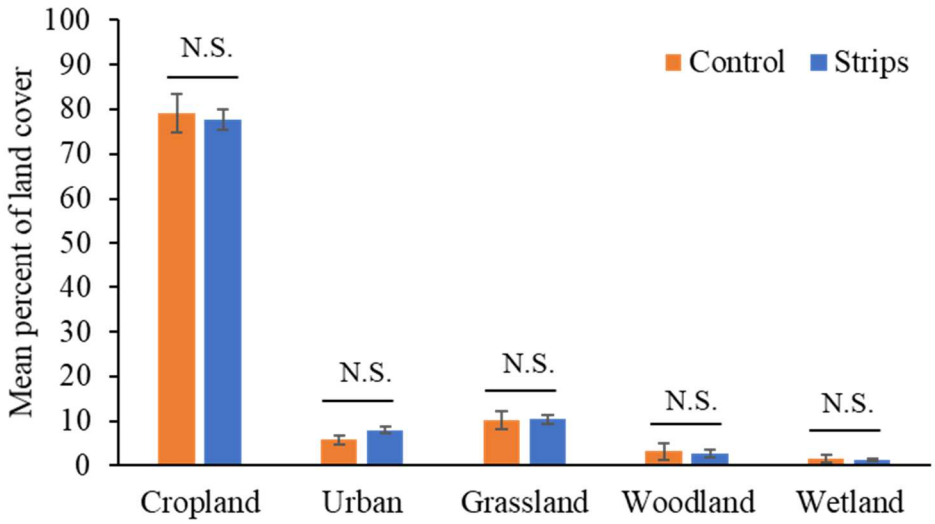


Figure 1. Land covers in the surrounding landscape within 1.6 km radius. N.S., no significant difference in any land cover between control and prairie strips sites ($P > 0.05$, *t* test referred to Supp. Table 5).

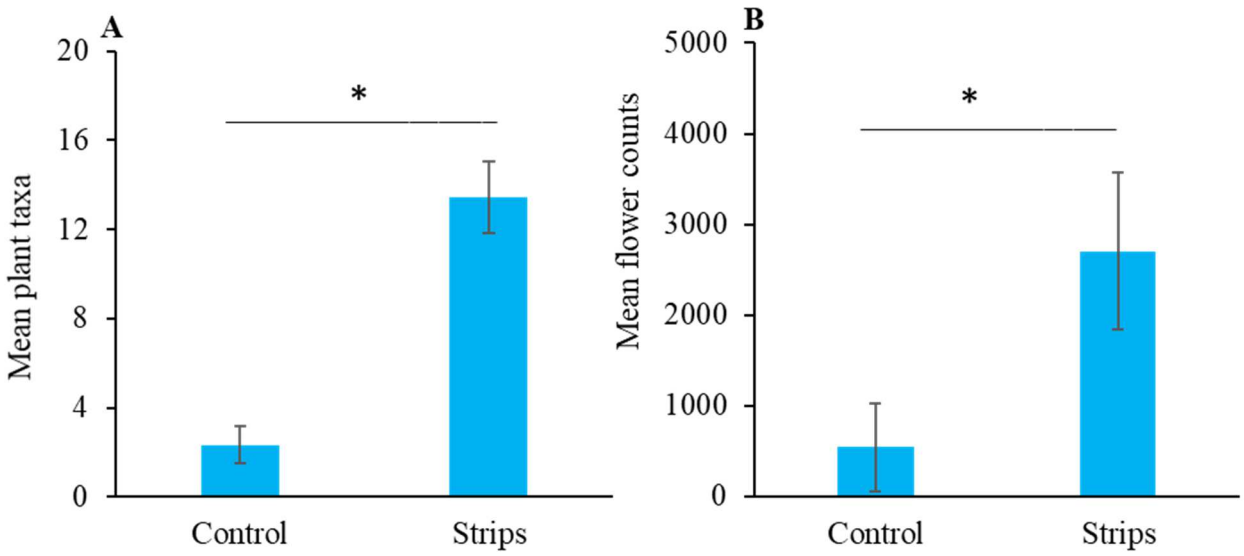


Figure 2. Mean flowering plant species (A) and flower counts (B) at control and prairie strips sites (n = 9 for both sites) from June through September during two years (2018-2019). Significant differences (*) were observed between control and prairie strips sites (mean plant taxa: $t = 6.08$, df = 112.11; $P < 0.0001$; mean flower counts: $t = 2.18$, df = 12.66; $P = 0.0485$).

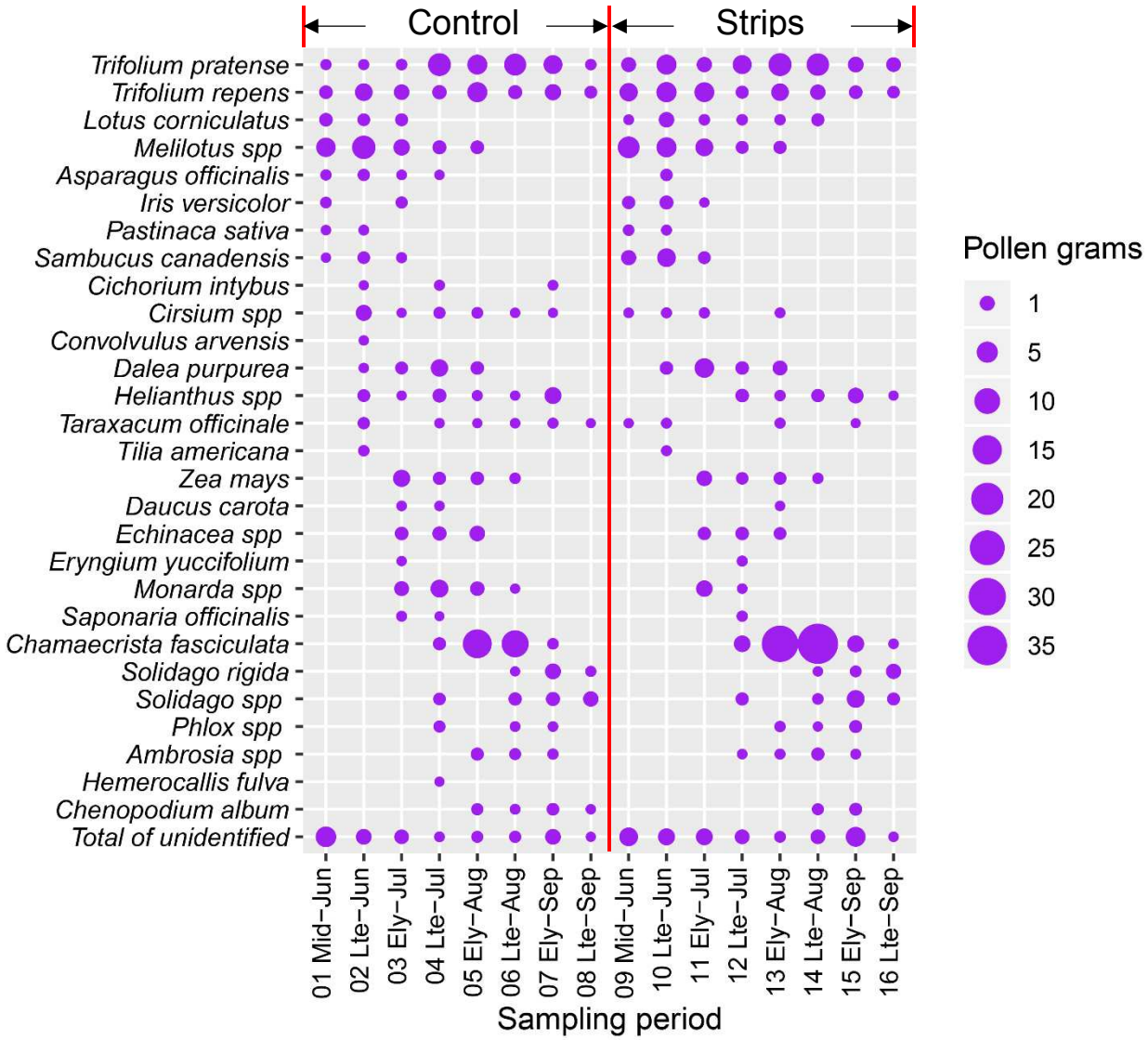


Figure 3. Grams of pollen by plant taxa collected per colony per apiary during each sampling period at control and prairie strips sites during 2017-2019. Unidentified pollen could not be assigned to a plant species were given a morphospecies name and recorded separately (referred to Supp. Table 6 for details of those morphospecies). Dates were organized by periods within a month, with specific dates found in Supp. Table 3: Ely = early, Mid = middle and Lte = late. *Helianthus* spp. in the plot represents a combination of three taxa, i.e. *Helianthus*, *Heliopsis & Silphium* spp. that were native sunflowers. *Monarda fistulosa* in the plot represents a combination of two species *Monarda fistulosa & PCnanthemum virginianum*.

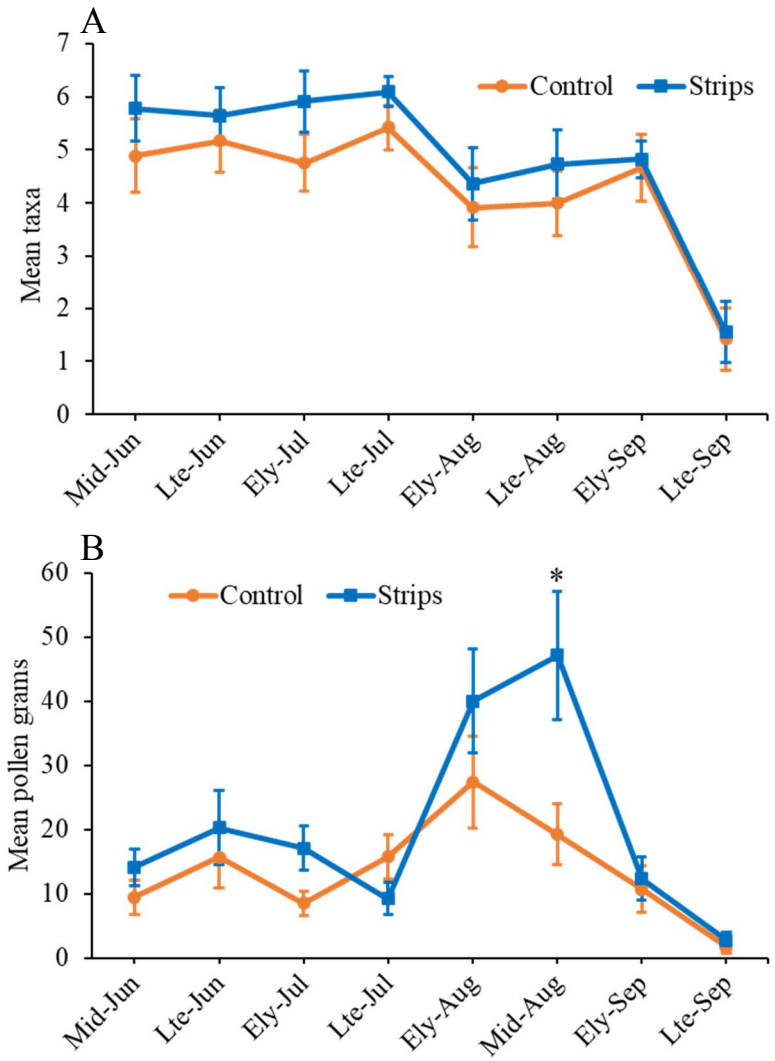


Figure 4. Diversity (A) and abundance (B) of pollen collected by honey bees on each sampling period at control and prairie strips sites (n = 11 for both). A repeated measures linear mixed ANOVA was used to analyze diversity and abundance across three years (2017-2019). The mean (± standard error) plant taxa found in pollen collected per apiary represents diversity. Pollen diversity did not differ significantly between control and strip sites ($F = 0.75$, df = 1, 37.7; $P = 0.3921$). The mean (± standard error) grams of pollen collected per apiary represent abundance. Overall, apiaries kept in prairie strips collected significantly more pollen than those at control sites ($F = 6.18$, df = 1, 50.3; $P = 0.0163$). At only one sampling period (Mid-Aug), significant

more pollen was collected by colonies at prairie strips using least squares under the model of mixed effect ANOVA (*; $P < 0.05$). Dates were organized by periods within a month, with specific dates found in Supp. Table 3: Ely = early, Mid = middle and Lte = late.

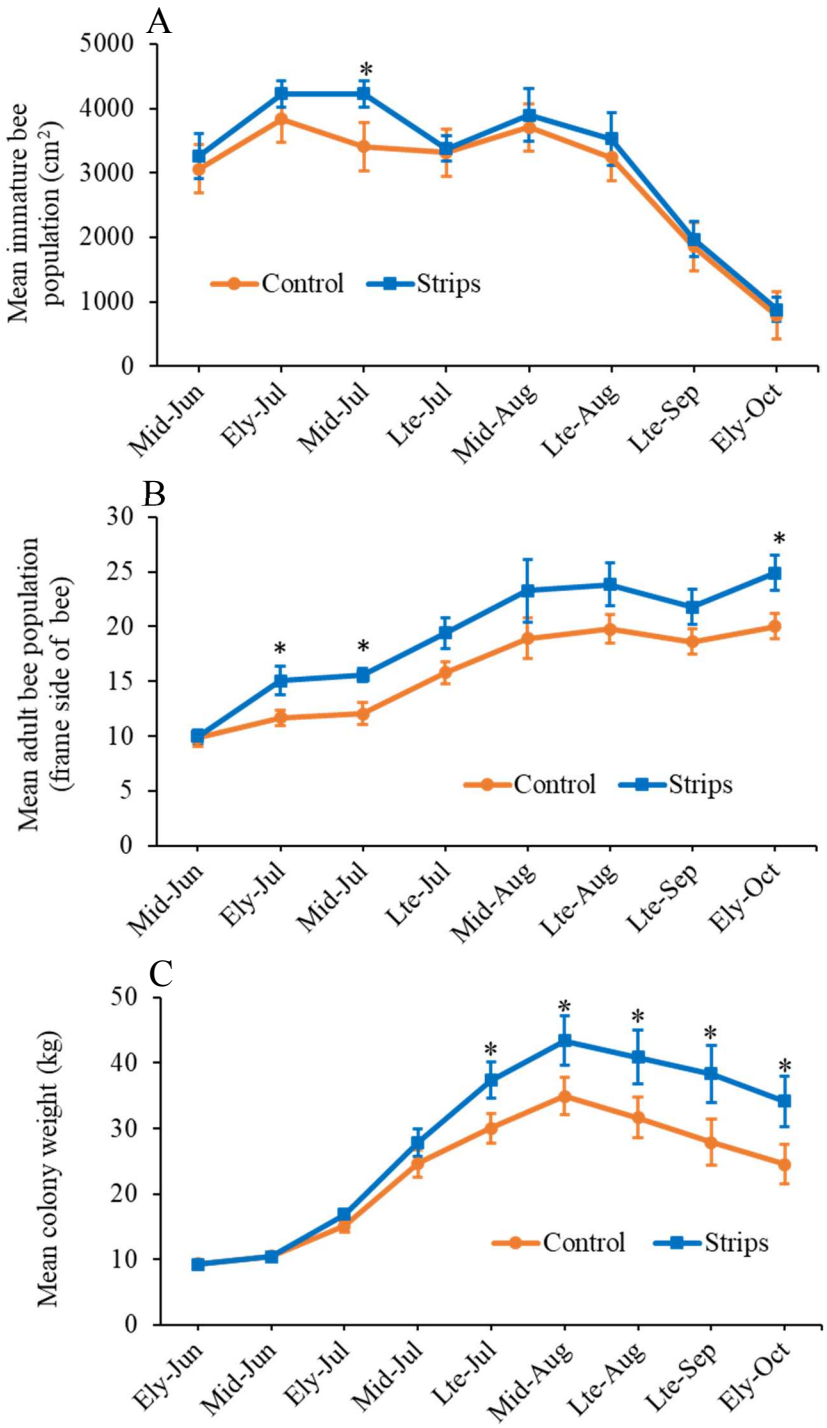


Figure 5. Populations of immature bees (A) and mature bees (B), and colony weight (C). Populations of immature and mature bees were measured during 2018 and 2019 (n = 9 for both control and prairie strips); while colony weight measured during 2017-2019 (n = 11 for both control and prairie strips,). Immature bee population were estimated based on the area of frames

covered by pupae (capped brood) per colony, and the mean (± standard error) did not differ between control and prairie strips sites ($F = 1.72$, df = 1, 7.83; $P = 0.2268$). Mature bee population were estimated based on the frame sides covered by adult bees per colony and did not differ between sites ($F = 6.43$, df = 1, 19.3; $P = 0.0199$). Colony weight includes all the biological material of a colony: bees, food stores (honey and pollen), wax. Mean colony weight differed between the two sets of treatment sites ($F = 4.54$, df = 1, 24.7, $P = 0.0432$). Symbol above each sampling period (*) indicates significant difference ($P < 0.05$) between two treatment sites for those three metrics using least squares within a mixed effect ANOVA with a repeated measures option. Dates were organized by periods within a month, with specific dates found in Supp. Table 3: Ely = early, Mid = middle and Lte = late.

Supplementary Tables and Figures

Supp. Table 1. Crops and coordinates of research sites in counties of Iowa during 2017-2019.

Year	Site name	County	Site type	Crop	Coordinate [a]
2017	JHN	Story	Control	Soybean	41.981244, -93.639407
2017	HRS	Story	Control	Soybean	42.107196, -93.581395
2017	GUT	Story	Strips	Corn	41.973874, -93.532388
2017	WOR	Story	Strips	Soybean	42.000478, -93.693143
2018	GUT	Story	Strips	Soybean	41.973874, -93.532388
2018	SME	Webster	Strips	Soybean	42.410917, -94.142687
2018	SMI	Wright	Strips	Corn	42.669853, -93.815655
2018	STN	Tama	Strips	Soybean	42.181555, -92.492799
2018	HAR	Story	Control	Soybean	41.947903, -93.473139
2018	JER	Tama	Control	Corn	42.702383, -93.859852
2018	KOE	Webster	Control	Soybean	42.548006, -93.992435
2018	HER	Tama	Control	Soybean	42.123310, -92.495442
2019	GUT	Story	Strips	Corn	41.973874, -93.532388
2019	SME	Webster	Strips	Corn	42.410917, -94.142687
2019	SMI	Wright	Strips	Soybean	42.669853, -93.815655
2019	STN	Tama	Strips	Corn	42.181555, -92.492799
2019	WOR	Story	Strips	Corn	42.000464, -93.693758
2019	HAR	Story	Control	Corn	41.947903, -93.473139
2019	JER	Tama	Control	Soybean	42.702383, -93.859852
2019	KOE	Webster	Control	Corn	42.548006, -93.992435

Supp. Table 1. Continued.

Year	Site name	County	Site type	Crop	Coordinate [a]
2019	HER	Tama	Control	Corn	42.123310, -92.495442
2019	DAI	Story	Control	Corn	41.977305, -93.658718

[a] Coordinate of the site also indicated the location of apiary at prairie strips or field margins for control sites.

Supp. Table 2. Information about sites installed with prairie strips.

Site name	No. of strips of prairie per site	Width/length of prairie strips (m) [a]	Area of prairie strips (hectare) [b]	Farm size (hectare) [b]	% of farm converted to Strips	Establish time	Burning in 2017-2019	Mowing in 2017-2019
GUT	4	5-8/207-412	2.14	25.50	8.41	2014	Fall of 2018, spring of 2019 [c]	No mowing
SME	2	8-26/245	0.77	23.47	3.28	2014	No burning	No mowing
SMI	3	32-35/840	4.47	81.01	5.51	2015	April of 2018	No mowing
STN	6	6-10/650	2.23	14.97	14.86	2016	April of 2018	No mowing
WOR[d]	5	6-12/309-380	0.85	11.74	7.24	2015	March of 2018	No mowing

[a] Width and length range of prairie strips was measured by Ruler-Path function of Google Erath Pro (Mountain View, CA).

[b] Size of total prairie strips in hectare at each site and farm size was provided by farmers.

[c] Around 50 % arear of prairie strips in GUT were burned in fall of 2018; while, the rest 50 % area burned in spring of 2019.

[d] The spots with Canada thistle (*Cirsium arvense*) were treated with herbicide in Wor in April of 2018 before we moved our colonies into the sites. We assumed that the herbicide treatment did not affect the health of our colonies.

Supp. Table 3. Data collection or sampling time during 2017-2019.

Year	Apiary inspection date [a] (assigned sampling period) [b]	Pollen collection date (assigned sampling period)	Plant survey
2017	May 2 (early), 26 (late)		
	Jun 22 (late)	Jun 3 (mid) [c], 23 (late)	
	Jul 7 (early), 21 (late)	Jul 7 (early), 25 (late)	
	Aug 2 (mid) [c], 23 (late)	Aug 4 (early), 23 (late)	
	Sep 5 (early), 25 (late)	Sept 5 (early), 16 (mid), 25 (late)	
2018	Jun 5 (early), 20 (late)	Jun 13 (mid), 28 (late)	Jun 17
	Jul 9 (early), 18 (mid), 31 (late)	Jul 11 (early) [c], 27 (late)	Jul 17
	Aug 15 (mid), 29 (late)	Aug 11 (early) [c], 28	Aug 7
	Sep 20 (late)	Sept 7 (early), 28 (late)	
	Oct 2 (early)		
2019	Jun 3 (early), 20 (late)	Jun 11 (mid), 25 (late)	Jun 5, 20
	Jul 2 (early), 18 (mid), 31(late)	Jul 9 (early), 24 (late)	Jul 15
	Aug 14 (mid), 28 (late)	Aug 6 (early), 23 (late)	Aug 14
	Sep 26 (late)	Sep 6 (early)	Sep 18
	Oct 9 (early)	Oct 4 (late-Sep) [c]	

[a] During apiary inspection, we collected data of colony weight, brood and adult bee population, queen presence and Varroa mite population, and sample of nurse bees for measuring lipid content in bee body.

[b] Each month was divided into three sampling periods, including early, middle and late, and data collection or sampling date was assigned to those three sampling periods. The criteria of assigning

a data collection or sampling date was to make each sampling period replicated as many as years, up to three years to increase the power of statistical analysis. In the main manuscript, the sampling periods that were replicated at least two years were represented in figures. This assignment was applied to apiary inspection and pollen collection, separately.

[c] Based on our criteria of the assignment of the sampling period described above, those data collection and sampling date that were only replicated for one year were switched to a nearest sampling period that had been replicated.

Supp. Table 4. Comparison of land covers in landscapes surrounding apiaries between control and prairies strips sites.

Land cover	t ratio	df	P > |t|
Cropland	-0.3048	15.03144	0.7647
Urban	1.947775	17.44921	0.0677
Grassland	0.034723	14.74417	0.9728
Woodland	-0.26556	13.31971	0.7946
Wetland	-0.39296	12.30447	0.7011

Supp. Table 5. Mean percent of pollen collected by each apiary across seasons during 2017-2019.

Plant taxa represented in bee pollen	Presence of plants [b]	Nativeness[c]	Control	Prairie strips
Ambrosia spp.	N/A	Native	0.55 ± 0.28	0.29 ± 0.15
Asparagus officinalis	N/A	Nonnative	0.29 ± 0.19	0.23 ± 0.21
Chamaecrista fasciculata	PS	Native	22.24 ± 6.21	35.7 ± 7.08
Chenopodium album	N/A	Nonnative	0.51 ± 0.31	0.34 ± 0.15
Cichorium intybus	C	Nonnative	0.24 ± 0.21	0
Cirsium spp.	PS	Nonnative	1.73 ± 0.95	0.13 ± 0.05
Convolvulus arvensis	C, PS	Nonnative	0.01 ± 0.01	0
Dalea purpurea	C, PS	Native	4 ± 2.36	3.09 ± 1.23
Daucus carota	C	Nonnative	0.09 ± 0.07	0.01 ± 0.01
Echinacea spp.	PS	Native	2.5 ± 1.01	0.78 ± 0.3
Eryngium yuccifolium	PS	Native	0.03 ± 0.03	0.04 ± 0.04
Helianthus, Heliopsis & Silphium spp.	PS	Native	2.91 ± 1.23	2.59 ± 0.85
Iris versicolor	N/A	Native	0.25 ± 0.17	0.66 ± 0.36
Lotus corniculatus	PS	Nonnative	1.01 ± 0.75	1.39 ± 1.2

Supp. Table 5. Continued.

Plant taxa represented in bee pollen	Presence of plants [b]	Nativeness[c]	Control	Prairie strips
Melilotus spp.	PS	Nonnative	11.48 ± 5.11	8.41 ± 2.69
Monarda fistulosa & PCnanthemum virginianum	PS	Native	4.35 ± 3.1	1.35 ± 0.88
Pastinaca sativa	C, PS	Nonnative	0.04 ± 0.03	0.08 ± 0.04
Phlox spp.	C	Native	0.16 ± 0.1	0.47 ± 0.28
Sambucus canadensis	N/A	Native	0.42 ± 0.23	2.2 ± 1.24
Saponaria officinalis	N/A	Nonnative	0.03 ± 0.03	0.06 ± 0.06
Solidago rigida	PS	Native	1.5 ± 0.82	0.73 ± 0.39
Solidago spp.	PS	Native	4.23 ± 2.47	3.69 ± 2.79
Taraxacum officinale	PS	Nonnative	0.26 ± 0.19	0.1 ± 0.06
Tilia americana	N/A	Native	0.08 ± 0.05	0.02 ± 0.02
Trifolium pratense	C, PS	Nonnative	19.92 ± 5.91	15.52 ± 4.51
Trifolium repens	C, PS	Nonnative	10.87 ± 2.86	11.05 ± 2.46
Zea mays	C, PS	Nonnative	2.98 ± 1.18	1.39 ± 0.68

Supp. Table 5. Continued.

Plant taxa represented in bee pollen	Presence of plants [b]	Nativeness[c]	Control	Prairie strips
UN-2 [a]		N/A	1.79 ± 1.22	0.51 ± 0.43
UN-4		N/A	0.0 2± 0.02	0.05 ± 0.04
UN-6		N/A	0.79 ± 0.67	0.1 ± 0.08
UN-9		N/A	0.64 ± 0.39	0.26 ± 0.13
UN-12		N/A	0.08 ± 0.03	0.24 ± 0.17
UN-16		N/A	0.01 ± 0.01	0.1 ± 0.08
UN-17		N/A	0.15 ± 0.15	0.19 ± 0.19
UN-22		N/A	0	0.03 ± 0.03
UN-23		N/A	0	0.22 ± 0.22
UN50		N/A	0	0.23 ± 0.19
UN51		N/A	0	0.15 ± 0.12
UN52		N/A	0	0.02 ± 0.02
UN53		N/A	0	0.03 ± 0.03
UN54		N/A	0	0.08 ± 0.08

Supp. Table 5. Continued.

Plant taxa represented in bee pollen	Presence of plants [b]	Nativeness[c]	Control	Prairie strips
UN56		N/A	0	0.02 ± 0.02
UN-100		N/A	1.61 ± 0.67	2.18 ± 1.14
UN-102		N/A	0.15 ± 0.08	0.23 ± 0.09
UN-103		N/A	0.08 ± 0.05	0.25 ± 0.25
UN-104		N/A	0	0.05 ± 0.03
UN-105		N/A	0	0.02 ± 0.02
UN-106		N/A	0.3 ± 0.19	0.15 ± 0.11
UN-107		N/A	0.03 ± 0.02	0
UN-110		N/A	0	0 [d]
UN-111		N/A	0.16 ± 0.13	3.2 ± 2.87
UN-112		N/A	0	0.02 ± 0.01
UN-113		N/A	0.08 ± 0.08	0.17 ± 0.11
UN-114		N/A	0.04 ± 0.04	0
UN-115		N/A	0	0.59 ± 0.44

Supp. Table 5. Continued.

Plant taxa represented in bee pollen	Presence of plants [b]	Nativeness[c]	Control	Prairie strips
UN-116		N/A	0.01 ± 0.01	0
UN-117		N/A	0.05 ± 0.04	0
UN-118		N/A	0	0.13 ± 0.13
UN-119		N/A	0.02 ± 0.02	0.11 ± 0.11
UN-120		N/A	0	0.03 ± 0.02
UN-121		N/A	0.13 ± 0.13	0
UN-200		N/A	0	0.04 ± 0.04
UN-201		N/A	0.01 ± 0.01	0.19 ± 0.13
UN-202		N/A	0.17 ± 0.17	0
UN-203		N/A	0.12 ± 0.1	0
UN-204		N/A	0.04 ± 0.04	0
UN-205		N/A	0.01 ± 0.01	0
UN-206		N/A	0	< 0.01
UN-207		N/A	0	0.07 ± 0.07

Supp. Table 5. Continued.

Plant taxa represented in bee pollen	Presence of plants [b]	Nativeness[c]	Control	Prairie strips
UN-208		N/A	0.01 ± 0.01	0.01 ± 0.01
UN-209		N/A	0.84 ± 0.84	0

[a] "UN-number" indicates the unidentified plant taxa collected by honey bees for pollen forage.

[b] "C" and "PS" were used to indicate if the plant taxa was present at control and prairie strips sites, respectively. "N/A" indicates the plants were neither found at controls nor prairie strips.

[c] "N/A" under nativeness column indicates that the unidentified plant taxa were assigned to native or nonnative category.

Supp. Table 6. Analysis of the impact of prairie strips on honey bee pollen forage and health using repeated measures linear mixed model.

Response variables	Transformation[a]	Effect	Num DF[b]	Den DF[b]	F Value	Pr > F
Pollen diversity	N/A	Strips	1	37.7	0.75	0.3921
		Year	2	38.2	2.56	0.0901
		Period[c]	7	125	12.93	<.0001
		Crop	1	37.6	0.16	0.6932
		Year × Strips	7	125	0.3	0.9543
		Period × Strips	2	38.3	0.23	0.7928
Pollen abundance	Square root	Strips	1	50.3	6.18	0.0163
		Period	7	129	14.61	<.0001
		Period × Strips	7	129	1.94	0.0688
Immature bee population	N/A	Strips	1	25.8	3.23	0.0839
		Year	1	26.4	3.36	0.078
		Period	7	90.6	34.77	<.0001
		Crop	1	27.7	0.22	0.6414

Supp. Table 6. Continued.

Response variables	Transformation[a]	Effect	Num DF[b]	Den DF[b]	F Value	Pr > F
		Year × Period × Strips	14	92.5	4.21	<.0001
		Year × Strips	1	25.8	0.08	0.7754
		Period × Strips	7	90.6	0.88	0.5264
Mature bee population	Log_{10}	Strips	1	19.3	6.43	0.0199
		Year	1	19.5	0.61	0.4457
		Period	7	95.1	25.27	<.0001
		Crop	1	20	0.54	0.4702
		Year × Period × Strips	14	95.4	2.92	0.001
		Year × Strips	1	19.3	3.4	0.0806
		Period × Strips	7	95.1	1.06	0.3957
Colony weight	Log_{10}	Strips	1	24.7	4.54	0.0432
		Year	2	25.3	21.38	<.0001
		Period	12	141	67.37	<.0001
		Crop	1	24.4	0.12	0.731

Supp. Table 6. Continued.

Response variables	Transformation[a]	Effect	Num DF[b]	Den DF[b]	F Value	Pr > F
		Year × Strips	2	25.3	1.35	0.2775
		Period × Strips	12	141	0.9	0.5506
Lipid content	Log_{10}	Strips	1	22.2	1.05	0.3167
		Year	1	21.1	99.62	<.0001
		Period	2	28.1	98.68	<.0001
		Crop	1	19.2	0.48	0.4959
		Year × Period × Strips	4	29	31.39	<.0001
		Year × Strips	1	22.2	0.67	0.4218
		Period × Strips	2	28.1	1.54	0.2314
Varroa population	Square root	Strips	1	15.6	0.34	0.5657
		Year	1	15.6	16.67	0.0009
		Month	2	27	18.41	<.0001
		Year × Period × Strips	4	27.6	5.46	0.0023
		Year × Strips	1	15.6	0.34	0.5706

Supp. Table 6. Continued.

Response variables	Transformation[a]	Effect	Num DF[b]	Den DF[b]	F Value	Pr > F
		Period × Strips	2	27	0.19	0.8314

[a] The value of response variables were transformed with $\log_{10}$ and square root to make the data conform to normal distribution.

[b] The degree freedom forms numerator and denominator.

[c] Sampling period.

Supp. Table 7. Analysis of the impact of prairie strips on honey bee pollen abundance with interaction with year, crop and interaction of year and sitetype using repeated measures linear mixed model.

Response variables	Transformation	Effect	Num DF	Den DF	F Value	Pr > F
Pollen abundance	Square root	Sitetype	1	38.3	5.21	0.0281
		Year	2	37.9	0.56	0.5784
		Period	7	107	17.73	<.0001
		Crop	1	39.2	2.9	0.0964
		Year × Sitetype	2	37.6	0.64	0.5314
		Period × Sitetype	7	107	2.6	0.0163

Supp. Table 8. The analysis of the effect of prairies strips on pollen diversity and abundance at each individual sampling period using post hoc *t* test based on difference of least square means of linear mixed effect models.

Response variables	Period	Sitetype	Sitetype	Estimate	Standard error	DF	t Value	Pr > |t|
Pollen diversity	Mid-Jun	Control	Prairie strips	-1.033	0.9225	129	-1.12	0.2649
	Late-Jun	Control	Prairie strips	-0.09172	0.8134	121	-0.11	0.9104
	Early-Jul	Control	Prairie strips	-0.819	0.8134	121	-1.01	0.316
	Late-Jul	Control	Prairie strips	-0.2735	0.8134	121	-0.34	0.7372
	Early-Aug	Control	Prairie strips	-0.1826	0.8134	121	-0.22	0.8227
	Late-Aug	Control	Prairie strips	-0.4554	0.8134	121	-0.56	0.5767
	Early-Sep	Control	Prairie strips	0.181	0.8134	121	0.22	0.8243
	Late-Sep	Control	Prairie strips	-0.09172	0.8134	121	-0.11	0.9104
Pollen abundance	Mid-Jun	Control	Prairie strips	-0.676	0.8372	140	-0.81	0.4208
	Late-Jun	Control	Prairie strips	-0.5444	0.7356	136	-0.74	0.4605
	Early-Jul	Control	Prairie strips	-1.0606	0.7356	136	-1.44	0.1516
	Mid-Jul	Control	Prairie strips	0.949	0.7356	136	1.29	0.1992
	Early-Aug	Control	Prairie strips	-0.964	0.7356	136	-1.31	0.1922

Supp. Table 8. Continued.

Response variables	Period	Sitetype	Sitetype	Estimate	Standard error	DF	t Value	Pr > |t|
	Mid-Aug	Control	Prairie strips	-2.3975	0.7356	136	-3.26	**0.0014**
	Early-Sep	Control	Prairie strips	-0.2502	0.7356	136	-0.34	0.7343
	Late-Sep	Control	Prairie strips	-0.2469	0.7356	136	-0.34	0.7377

Supp. Table 9. Comparison of pollen diversity between late September with any other period by post hoc t test using least square means.

Period	Period	Estimate	Standard error	DF	t Value	Pr > |t|
Mid-Jun	Late-Sep	3.7753	0.5973	130	6.32	<.0001
Late-Jun	Late-Sep	4.0909	0.5651	126	7.24	<.0001
Early-Jul	Late-Sep	4	0.565	127	7.08	<.0001
Late-Jul	Late-Sep	4.4545	0.5643	131	7.89	<.0001
Early-Aug	Late-Sep	2.7727	0.5606	141	4.95	<.0001
Late-Aug	Late-Sep	3	0.5447	151	5.51	<.0001
Early-Sep	Late-Sep	3.4091	0.4797	113	7.11	<.0001

Supp. Table 10. The analysis of the effect of prairies strips on colony weight at each individual sampling period using post hoc *t* test based on difference of least square means of linear mixed effect models for colony data pooled across 2017-2019.

Response variables	Period	Sitetype	Sitetype	Estimate	SE	DF	t Value	Pr > |t|
Immature bee	Mid-Jun	Control	Prairie strips	-0.2232	0.4383	94.8	-0.51	0.6118
population	Early-Jul	Control	Prairie strips	-0.4377	0.4383	94.8	-1	0.3204
	Mid-Jul	Control	Prairie strips	-0.9345	0.4383	94.8	-2.13	**0.0356**
	Late-Jul	Control	Prairie strips	-0.06876	0.4383	94.8	-0.16	0.8757
	Mid-Aug	Control	Prairie strips	-0.2124	0.4383	94.8	-0.48	0.629
	Late-Aug	Control	Prairie strips	-0.3263	0.4383	94.8	-0.74	0.4584
	Late-Sep	Control	Prairie strips	-0.3411	0.4383	94.8	-0.78	0.4383
	Early-Oct	Control	Prairie strips	-0.09577	0.4383	94.8	-0.22	0.8275
Mature bee population	Mid-Jun	Control	Prairie strips	-0.01644	0.1043	62.4	-0.16	0.8753
	Early-Jul	Control	Prairie strips	-0.2324	0.1043	62.4	-2.23	**0.0295**
	Mid-Jul	Control	Prairie strips	-0.2719	0.1043	62.4	-2.61	**0.0114**
	Late-Jul	Control	Prairie strips	-0.1917	0.1043	62.4	-1.84	0.0709
	Mid-Aug	Control	Prairie strips	-0.1803	0.1043	62.4	-1.73	0.0889

Supp. Table 10. Continued.

Response variables	Period	Sitetype	Sitetype	Estimate	SE	DF	t Value	Pr > |t|
	Late-Aug	Control	Prairie strips	-0.1653	0.1043	62.4	-1.58	0.1181
	Late-Sep	Control	Prairie strips	-0.14	0.1043	62.4	-1.34	0.1846
	Early-Oct	Control	Prairie strips	-0.2013	0.1043	62.4	-1.93	**0.0582**
Colony weight	Early-May	Control	Prairie strips	0.07719	0.1521	156	0.51	0.6125
	Early-Jun	Control	Prairie strips	0.02198	0.09257	59	0.24	0.8131
	Mid-Jun	Control	Prairie strips	0.01653	0.09628	68.3	0.17	0.8642
	Late-Jun	Control	Prairie strips	0.03842	0.1435	157	0.27	0.7892
	Early-Jul	Control	Prairie strips	-0.09522	0.09257	59	-1.03	0.3078
	Mid-Jul	Control	Prairie strips	-0.1064	0.09529	66	-1.12	0.2683
	Late-Jul	Control	Prairie strips	-0.1923	0.09257	59	-2.08	**0.0421**
	Early-Aug	Control	Prairie strips	-0.3094	0.1435	157	-2.16	**0.0325**
	Mid-Aug	Control	Prairie strips	-0.1571	0.09627	68.3	-1.63	0.1074 [a]
	Late-Aug	Control	Prairie strips	-0.2121	0.09257	59	-2.29	**0.0255**
	Early-Sep	Control	Prairie strips	-0.2966	0.1335	151	-2.22	**0.0278**

Supp. Table 10. Continued.

Response variables	Period	Sitetype	Sitetype	Estimate	SE	DF	t Value	Pr > |t|
	Late-Sep	Control	Prairie strips	-0.258	0.09257	59	-2.79	**0.0071**
	Early-Oct	Control	Prairie strips	-0.287	0.0983	68.7	-2.92	**0.0047**
Lipid percentage	Mid-Jun	Control	Prairie strips	-0.03921	0.04163	39	-0.94	0.3521
	Mid-Aug	Control	Prairie strips	0.04802	0.03977	38.7	1.21	0.2345
	Early-Oct	Control	Prairie strips	0.05377	0.03977	38.7	1.35	0.1842
Varroa mite population	Jun	Control	Prairie strips	-0.08287	0.1419	40.5	-0.58	0.5624
	Jul	Control	Prairie strips	0.0106	0.1419	40.5	0.07	0.9408
	Aug	Control	Prairie strips	-0.08523	0.1419	40.5	-0.6	0.5514

[a] Colony weight was only replicated for one year, i.e. at middle August of 2017. This results was not indicted in figure 5 of the main manuscript that only demonstrated the results replicated for at least two years.

Supp. Table 11. Multiple comparisons of colony weight among years (2017-2019) with Tukey-Kramer adjustment.

Year	Year	Estimate	SE	df	t Value	Pr > |t|	Adjusted P value
2017	2018	0.3822	0.08295	26.1	4.61	<.0001	0.0003
2017	2019	-0.055	0.08889	25.8	-0.62	0.5416	0.8114
2018	2019	-0.4372	0.07314	24.4	-5.98	<.0001	<.0001

Supp. Table 12. Multiple comparisons of percent of lipid in nurse bees among three sampling periods in 2018-2019 with Tukey-Kramer adjustment.

Period[a]	Period	Estimate	Standard	DF	t Value	Pr > |t|	Adjust P value
Mid-Jun	Mid-Aug	0.29	0.03123	24.2	9.29	<.0001	<.0001
Mid-Jun	Early-Oct	-0.1393	0.02874	39.3	-4.85	<.0001	0.0001
Mid-Aug	Early-Oct	-0.4293	0.03062	23.3	-14.02	<.0001	<.0001

[a] Lipid content were monitored in various periods of 2018 and 2019.

Supp. Table 13. Multiple comparisons of Varroa mite populations among three months in 2018-2019 with Tukey-Kramer adjustment.

Period (month)	Period (month)	Estimate	Standard	DF	t Value	Pr > |t|	Adjust P value
Jun	Jul	-0.1395	0.09241	24.2	-1.51	0.1441	0.3023
Jun	Aug	-0.5823	0.101	41.6	-5.76	<.0001	<.0001
Jul	Aug	-0.4428	0.09241	24.2	-4.79	<.0001	0.0002

Supp. Table 14. Estimation of difference of honey weight per colony between control and prairie strips when colony weight is at its peak, i.e. middle August.

Difference of mean colony weight [a]	Difference of mean weight of immature bee [b]	Difference of mean weight from adult bees [d]	Difference of mean pollen weight [c]	Difference of mean wax weight [d]	Difference of mean honey weight [e]	Contribution of honey to colony weight in prairie strips [f]
8.43 kg	0 kg	1.12 kg	0 kg	0 kg	7.31 kg	87 %

[a] The difference of mean colony weight between control and prairie strips. The colony weight include the weight from immature bee, mature bees, pollen and honey within a colony.

[b] Because the brood population between control and prairie strips were similar, we assumed the weight from immature were same resulting into to no difference in weight of immature bees.

[c] We assumed a frame side of bee contained 2, 000 individual adults that fully cover one side of a frame.

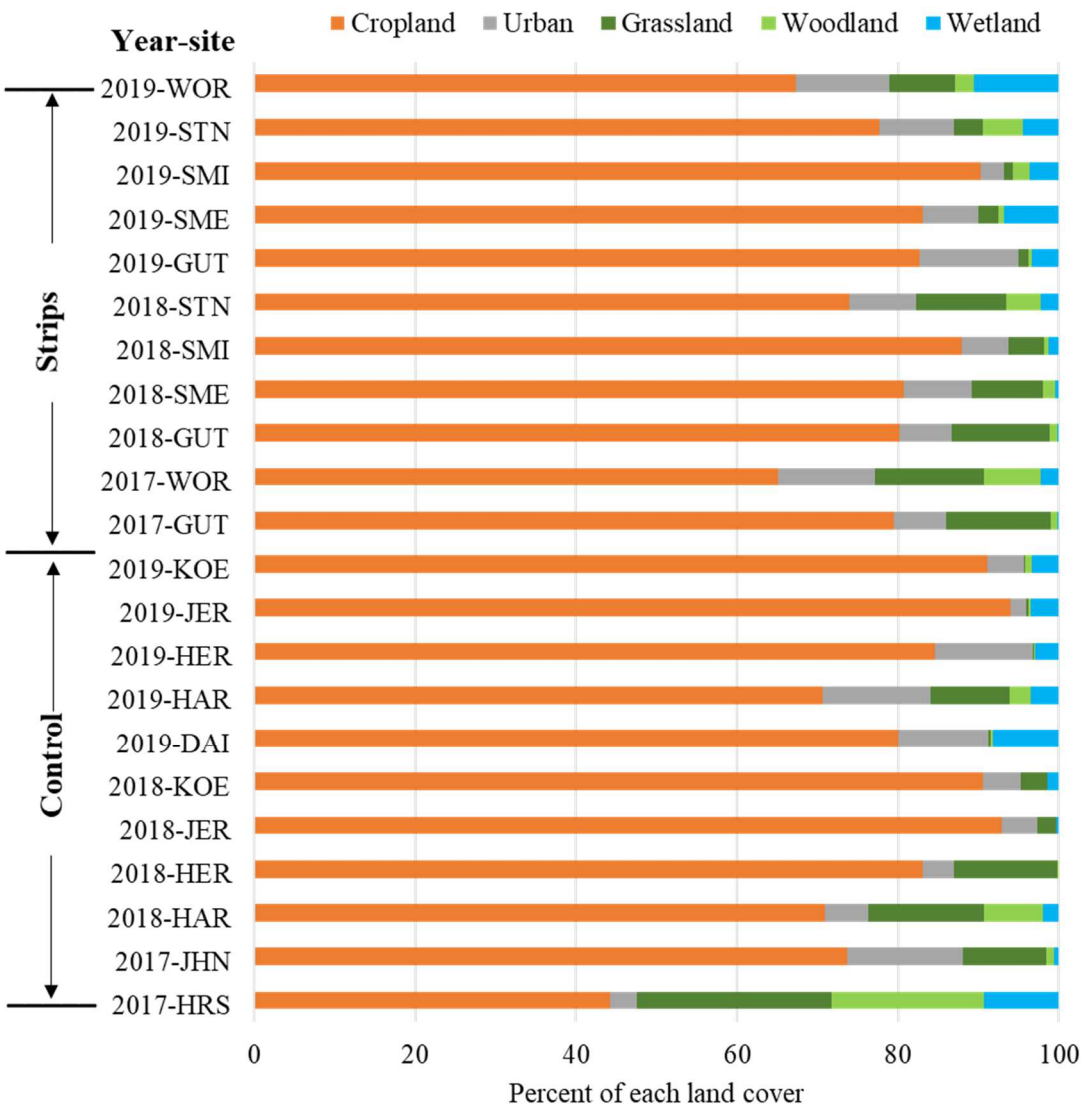
[d] We assumed 7, 700 adults bees weighed as 1 kg (3500 adult bees as 1 pound in USA).

[c] Though colonies at prairie strips collected more pollen than those at control sites, the pollen contributed to a very small fraction of total colony weight. We assumed the difference of mean pollen weight were zero for convenience of estimating the difference of mean honey weight.

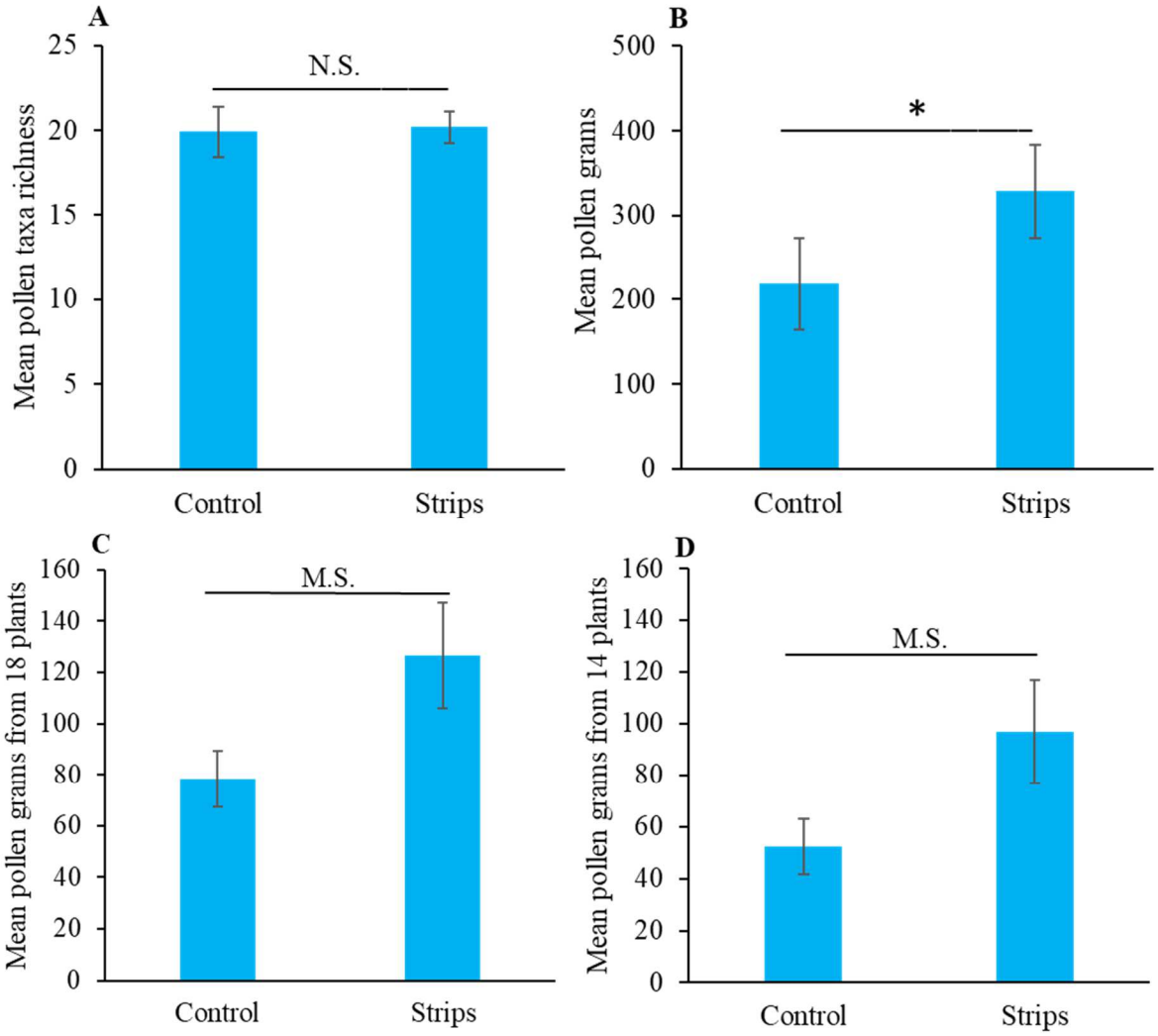
[d] We did not measure the wax weight in the colony. Because wax weight was a small fraction of colony weight and we neglected the potential difference in wax weight between control and prairie strips.

[e] Difference of mean honey weight = difference of mean colony weight – difference of mean weight from adult bees

[f] Contribution of honey to colony weight at prairie strips = (difference of mean honey weight ×100) / Difference of mean colony weigh.

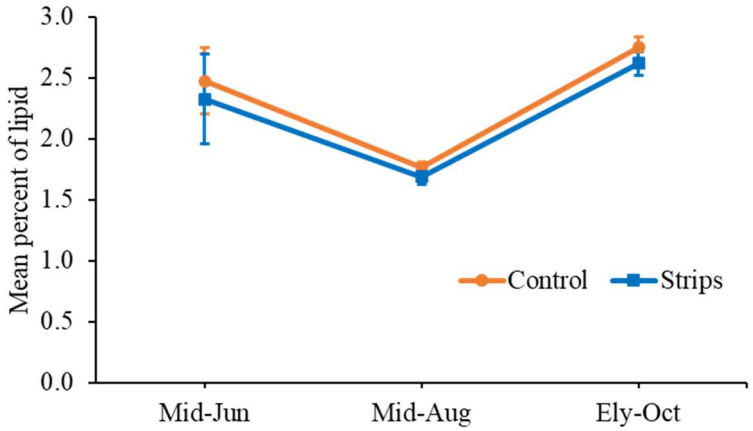


Supp. Figure 1. Percent of land covers within the landscapes surrounding apiaries at 1.6 km radius.

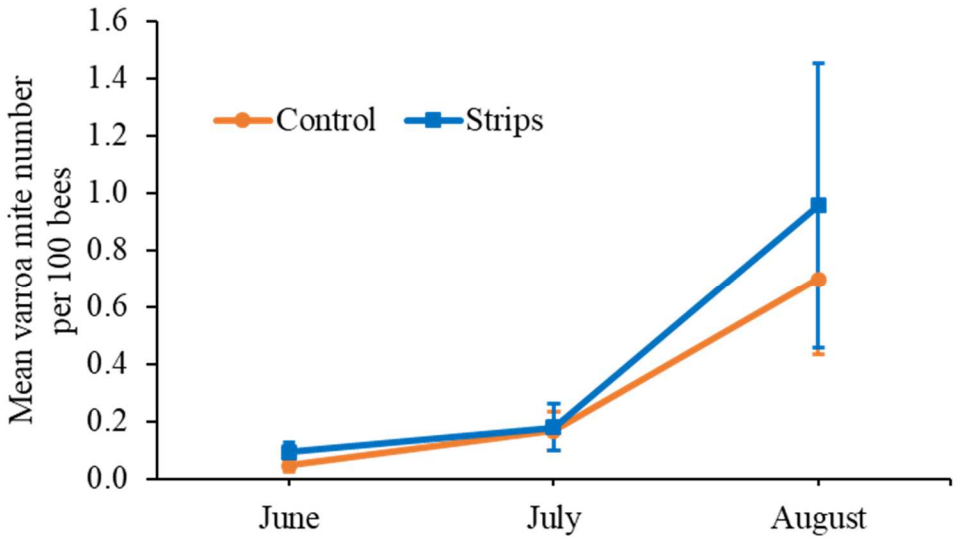


Supp. Figure 2. A) mean total plant taxa found in pollen collected by each apiary across seasons. N.S., no significant difference between control and prairie strips sites ($t = 0.16$, df = 16.86; $P = 0.87$). B) mean total amount of pollen (g) collected by each colony across the seasons. Mean total plant grams were significant difference (*) between control and prairie strips ($t = 2.31$, df = 16.32; $P = 0.0342$). C) mean total amount of pollen (g) collected from 18 flowering plant taxa per colony across seasons. Those 18 flowering plants could be found in prairie strips. M.S., marginal significance between control and prairie strips ($t = 2.08$, df = 12.17; $P = 0.058$). D) mean total amount of pollen (g) collected from 14 flowering plant taxa per colony across seasons. Those 14 plant taxa were unique to prairie strips that can not be found at control sites.

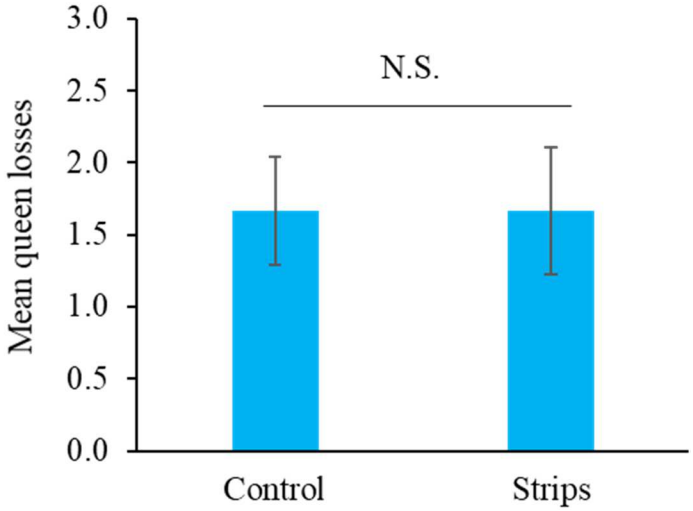
M.S., marginal significance between control and prairie strips (t = 1.95, df = 12.17; P = 0.0746). Plots A and B were made based on the pollen data obtained in 2017-2019 (replications for both control and prairie strips = 11). Plots C and D were made based on the pollen data obtained in 2018 and 2019 when plant survey were conducted (replications for both control and prairie strips = 9).



Supp. Figure 3. Percent of lipid content in nurse bees in each sampling period. Repeated measures linear mixed effect ANOVA was used for statistical analysis upon data collected in two years (2018-2019). Percent of lipid content (Mean ± SE) in nurse bees did not differ between control and prairie strips sites (n = 9 for control and prairie strips sites, $F = 1.05$, df = 1, 22.2; $P = 0.3167$).



Supp. Figure 4. Varroa mite populations in each month. Repeated measures linear mixed effect ANOVA was used for statistical analysis upon data collected in two years (2018-2019). Varroa mite population was represented by mean (± SE) Varroa mite number per 100 bees across colonies in each apiary. Varroa mite population did not differ between control and prairie strips site ($F = 0.34$, df = 1, 15.6; $P = 0.5657$).



Supp. Figure 5. Mean (± SE) number of queen losses of each apiary across the growing season. N.S., no significant difference in queen losses between control and prairie strips sites ($t = 0$, df = 15.57; $P = 1$).

CHAPTER 6. GENERAL CONCLUSIONS

Challenges to honey bee health have mounted in recent years, with the rise of human-driven global change, notably the expansion of industrial scale agriculture. The overall objective of this dissertation was to better understand the effects of variation in land use within an agricultural landscape and associated floral resources on the diversity and abundance of forage collected by and health of honey bees. Focusing on pollen (as bees' most important dietary source of macronutrients), I found little variation in pollen collection by bees across different types of agricultural landscapes and habitat types (low vs high cultivation landscapes, soybean monocultures vs diversified fruit and vegetable farms, and restored prairies). Restored prairie was an important source of pollen for the nonnative honey bee, and integration of small patches of prairie into cropland (namely, prairie strips) enhanced pollen availability and honey bee health.

The goal of **Chapter 2** was to determine whether low cultivation (lower percent of cropland) landscapes provide more diverse and abundant pollen forage to honey bees than high cultivation (higher percent of cropland) landscapes. Honey bee colonies located in soybean fields within both landscapes were monitored using pollen traps, followed by an estimation of pollen diversity and abundance. Contrary to my hypothesis, I found diversity and abundance of pollen did not differ between low versus high cultivation landscapes. In both landscape categories, leguminous plants, including native and nonnative species were the major pollen sources for honey bees in agricultural areas. This led to an important question: is the limited diversity of pollen types collected by bees in agricultural landscapes sufficient to support honey bee health? To address one aspect of this, I experimentally tested the effects of the most common pollen that bees collected in agricultural landscapes in central Iowa, examining whether this simple pollen

mix can provide nutritional health benefits that provides resistance to virus infection. I found that an addition of a second species of a leguminous plant partridge pea [*Chamaecrista fasciculata*]) to the pollen diet (i.e. clover [*Trifolium* spp.] and improved survival of honey bees infected with virus. These experiments provided useful knowledge about bees' pollen usage and its potential nutritional benefits in agriculturally intense landscapes.

The goal of **Chapter 3** was to explore whether more diverse landscape features (diverse fruit and vegetable farms and prairies) would provide more diverse and abundant pollen to honey bees than soybean farms. Pollen samples were collected from pollen traps placed on managed honey bee colonies located in soybean farms, diverse fruit and vegetable farms, and restored prairies, and pollen was identified and measured for taxonomic diversity and abundance. In addition, utilizing multi-year datasets from colonies located in soybean farms, I also assessed whether annual weather fluctuations could explain variation in diversity and abundance of pollen collected by honey bees. I found the diversity and abundance of pollen collected by honey bees did not differ among soybean farms, diverse fruit and vegetable farms, and prairies. Instead, climate conditions related to drought and high temperatures in July were associated with reduced pollen abundance. These conditions were also associated with higher pollen diversity, which may be due to the fact that forager bees need to scout a large area and forage on a wider variety of plants to compensate for an overall shortage in pollen.

The goal of **Chapter 4** was to understand how nonnative honey bees utilize restored native prairies as a source of pollen. My specific aim was to find which prairie plants are used by honey bees for pollen forage, and how forage abundance and composition collected in prairies changes during a growing season. Over a three-year period, using pollen traps on hives located within restored prairie sites, I found honey bees continuously collected pollen from prairie plants

throughout June to September. Importantly, prairie-derived pollen was collected more often in August and September when crops and weedy plants ceased blooming. These results emphasize the importance of seasonal variation in honey bee forage, and the potential for diverse native habitats to provide forage at times in the season when agricultural landscapes cannot.

The goal of **Chapter 5** was to determine in an on-farm setting if an agriculture practice, the use of prairie strips integrated into cropland, benefit honey bee health. I hypothesized that prairie strips would provide more diverse and abundant pollen forage and support healthier colonies across the growing season compared to cropland without prairie strips (control). I found floral resources were significantly more diverse and abundant at prairie strips than at control sites, and bees collected more pollen at prairie strips overall and also on specific dates. Importantly, bees utilized plants found in prairie strips; I found that colonies collect pollen from 50 % of the plant taxa (18 of 36 taxa) found growing in prairie strips. Fourteen of these plant taxa were uniquely found to be growing in prairie strips; this suggested prairie strips provided bees with ready access to sources of forage. In addition, I found adult bee populations were larger and colonies were heavier at prairie strips than control sites. As honey is the major contributor to colony mass in summer and fall, the fact that I found heavier colonies also suggested that there was more nectar available to honey bees at prairie strips. These results suggested that prairie strips significantly improved pollen and nectar availability and overall honey bee colony health. Overall, these results suggested very positive effects of prairie strips for honey beekeeping, however an important caveat is that apiary size was small (four colonies). Thus, small ("hobby" beekeeper sized) apiaries may benefit from prairie strips, but an important question for future research is to determine whether prairie strips have the capacity to support larger, commercial size apiaries.

Overall, my dissertation led to some novel general conclusions about honey bee nutritional health in agricultural landscapes. First, climate and seasonal effects appeared to be stronger determinants of pollen availability than landscape composition. Contrary to our expectations based on landscape diversity, row-crop fields within a low cultivation landscapes (with more non-crop land cover including woodland, urban, grassland and woodland), diverse fruit and vegetable farms and prairies did not enhance honey bee abundance and diversity of pollen collected by honey bees. However, locating honey bees in row-crop fields integrated with prairie strips where (especially late season) floral resources were enhanced improved abundance of bee-collected pollen and overall colony size. The combined results of these chapters suggest that hybrid landscapes consisting of immediately adjacent native prairie strips combined with large tracts of cropland provided a good balance of seasonally and taxonomically distributed floral resources that can benefit forage availability and overall health of honey bees.